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Natural Attenuation of Explosives in Soil and Water Systems at Department of Defense Sites: Interim Report

by *Judith C. Pennington, Douglas Gunnison, Danny W. Harrelson, James M. Brannon, Mansour Zakikhani, Thomas F. Jenkins, Joan U. Clarke, Charolett A. Hayes, Tommy Myers, Ed Perkins, David Ringelberg, Dan M. Townsend, Herbert Fredrickson, James H. May*

Prepared for Headquarters, U.S. Army Corps of Engineers

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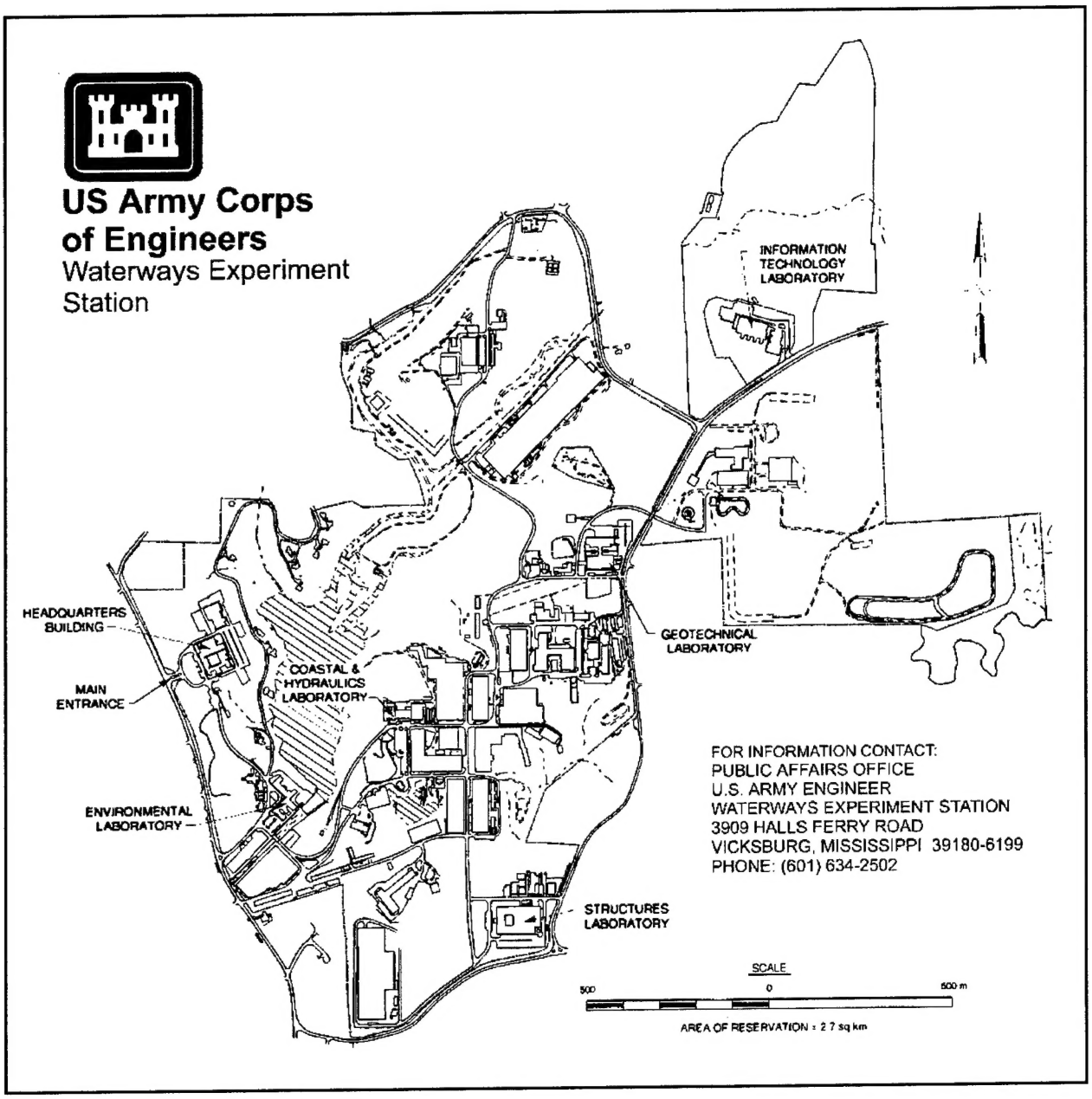

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Preface

The report herein was prepared by the Environmental Laboratory (EL) of the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, in association with the U.S. Army Cold Regions Research and Engineering Laboratory (CRREL), Hanover, NH, AScI Corporation, McLean, VA, and DynTel, Vicksburg, MS. WES is a complex of five laboratories of the U.S. Army Engineer Research and Development Center (ERDC). The research was sponsored by the Strategic Environmental Research and Development Program, Arlington, VA. Dr. John Harrison and Mr. Bradley P. Smith, Executive Directors, Project Number 1043, and the Environmental Security Technology Certification Program, Arlington, VA, Dr. Jeffrey Marqusee, Program Manager, Project Number 9518. The U.S. Army Industrial Operations Command, Rock Island, IL, Mr. Cyril Onewokae, Environmental Quality Directorate, funded monitoring at the validation site. The Principal Investigator was Dr. Judith C. Pennington, EL.

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Conversion Factors, Non-SI to SI Units of Measurement

Non-SI units of measurement used in this report can be converted to SI units as follows:

Multiply	By	To Obtain
acres	4,046.873	square meters
cubic yards	0.7645549	cubic meters
feet	0.3048	meters
gallons (U.S. liquid)	3.785412	liters
inches	2.54	centimeters
miles (U.S. statute)	1.609347	kilometers
square miles	2.589998	square kilometers
tons (2,000 pounds, mass)	907.1847	kilograms

1 Introduction

Background

Precedents

The regulatory community and the general public are increasingly aware that solutions to environmental contamination are not as simple as imposing stringent regulations. Environmental remediation technology is evolving toward more practical goals incorporating less expensive, less intrusive, long-term solutions. Natural attenuation may be a legitimate and sensible alternative to existing remediation techniques if appropriate implementation guidance is developed. A recent study verified a regulatory attitude of potential acceptance of natural attenuation for explosives-contaminated sites (Balasco et al. 1996). This study confirmed that most regulatory agencies would accept natural attenuation given appropriate scientific, engineering, and risk assessment data.

A significant precedent for natural attenuation of environmental contaminants has been set by the widely implemented protocol for natural attenuation of fuels developed by the Air Force Center for Environmental Excellence (AFCEE) (Wiedemeier et al. 1995a,b). The protocol has been implemented at more than 60 sites nationwide. The protocol espouses development of the following three lines of evidence for natural attenuation: (a) documented loss of contaminants at the field scale, (b) use of chemical analytical data in mass balance calculations, and (c) laboratory microcosm studies using aquifer samples collected from the site. To that end, the following eight steps are required:

a. Review existing site data.

b. Develop preliminary conceptual model for the site and assess potential significance of natural attenuation.

c. Perform site characterization in support of natural attenuation.

d. Refine conceptual model based on site-characterization data, complete premodeling calculations, and document indicators of natural attenuation.

e. Model natural attenuation using numerical fate and transport models that allow incorporation of a biodegradation term (e.g., Bioplume II or Bioplume III).

f. Conduct an exposure assessment.

g. Prepare long-term monitoring plan.

h. Present findings to regulatory agencies and obtain approval for the natural attenuation with long-term monitoring option.

The protocol relies appropriately upon indicators of microbiological degradation processes, which are the most significant degradation processes for fuels. The AFCEE has developed another protocol for chlorinated solvents that is being equally well received (Wiedemeier et al. 1996).

Because of the successes of these protocols and general concern for adoption of responsible remediation practices, the U.S. Environmental Protection Agency (EPA) Office of Solid Waste and Emergency Response (OSWER) has developed a policy directive to clarify EPA policy regarding the use of "monitored natural attenuation" at sites regulated by their office (EPA 1997). These sites include Superfund, RCRA Corrective Action, and Underground Storage Tanks sites. The directive emphasizes source control, monitoring, and use of "three lines of evidence," which are similar to those developed in the AFCEE protocol for fuels. The lines of evidence are as follows:

a. Historical groundwater and/or soil chemistry data that demonstrate a clear meaningful trend of declining contaminant mass and/or concentrations at appropriate monitoring or sampling points.

b. Hydrogeologic or geochemical data that can be used to indirectly demonstrate the type(s) of natural attenuation processes active at the site and the rate at which such processes will reduce contaminant concentrations to required levels.

c. Data from field or microcosm studies (conducted in or with actual contaminated site media) that directly demonstrate the occurrence of a particular natural attenuation process at the site and its ability to degrade the contaminants of concern (typically used to demonstrate biological degradation processes only).

The directive includes various "physical, chemical, or biological processes that, under favorable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater." The directive is risk-based and requires planning of "contingency remedies" should natural attenuation prove less effective than anticipated.

The Army has also circulated an interim policy statement on natural attenuation (*Federal Register* 1990). This statement mandates that natural attenuation be at least considered for all Army sites when developing remediation plans.

Fate processes of explosives

Contaminant introduction. Much of the explosives contamination of the environment has resulted from manufacturing and load-assemble-package (LAP) processes conducted during and before World War II and the Korean Conflict. Principal explosives waste products were 2,4,6-trinitrotoluene (TNT), 1,3,5-hexahydro-1,3,5-trinitrotriazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and N,2,4,6-tetranitro-N-methylaniline (tetryl). Environmental awareness had not yet developed, so waste-disposal practices were governed more by convenience and explosives safety concerns. Waste waters from manufacture and from LAP operations were often discharged into sumps, runoff and/or percolation ditches, and lagoons. These holding areas afforded opportunity for photolysis because of surface exposure to sunlight, for sedimentation of particulates, and for recrystalization and precipitation of solid products. Photodegradation products of TNT are frequently among the most prevalent contaminants in soils and groundwater on these sites. The principal photodegradation product of TNT is 1,3,5-trinitrobenzene (TNB), but occasional detections of dinitrobenzenes and nitrobenzene have been reported.

Transformation. Transformation is perhaps the single most significant environmental fate process for TNT. The monoamino products are frequently observed in the environment, the diamino less often, and the triamino not at all. The amino transformation products of TNT are potentially subject to further interactions with each other and with various components of natural systems. RDX and HMX transform microbially to mono- and di-nitroso products (McCormick, Cornell, and Kaplan 1985). These transformations occur anaerobically; only limited transformation under aerobic conditions have been reported (Spanggord et al. 1983; McCormick, Cornell, and Kaplan 1981,1985).

Microbial degradation. While readily transformed to the amino products, TNT is only slowly mineralized in soils and groundwater (Pennington et al. 1998). A mineralization pathway for TNT has been proposed (Duque et al. 1993; Preuss, Fimpel, and Diekert 1993). RDX, which is relatively unaffected by transformation and sequestration processes, is also more readily mineralized than TNT, especially under anaerobic conditions (McCormick, Cornell, and Kaplan 1985). Descriptions of pathways and rates for degradation of other energetics are limited. HMX biotransformation is limited to anaerobic conditions, is slower than degradation of RDX, and results in mono-, and di-nitroso intermediates (McCormick, Cornell, and Kaplan 1985). Tetryl has been postulated to transform to *N*-methylpicramide by loss of a nitro-group from the aniline moiety (Kaplan 1993). The structural similarity to TNT suggests that mineralization of tetryl may proceed slowly.

Immobilization. Evidence for immobilization of TNT in soils with consequent reduction in bioavailability has been dramatically illustrated in plant-uptake studies and in composting of TNT-contaminated soils (Folsom et al. 1988; Pennington 1988; Fellows, Harvey, and Cataldo 1993; Kaplan and Kaplan 1982; Pennington et al. 1995a; Pennington et al. 1997). Immobilization of RDX and other energetic contaminants is limited compared with immobilization of TNT. Partitioning of the energetics to soil particles is not a significant sequestration process and is often

reversible (Pennington and Patrick 1990; Pennington et al. 1995b; Myers et al. 1998; Ainsworth et al. 1993; Selim, Xue, and Iskandar 1995; Xue, Iskandar, and Selim 1995). However, transformation of TNT followed by partitioning and other interactions between transformation products and soils impacts transport potential and rate (Townsend and Myers 1996). Specific and reversible adsorption of nitroaromatic compounds, including explosives (e.g., TNT and TNB), to clays has been demonstrated (Haderlein, Weissmahr, and Schwarzenbach 1996; Price, Brannon, and Hayes 1997).

Objectives

The broad objectives of the project were to demonstrate natural attenuation of explosives at an Army site and to develop a protocol for selection and implementation of natural attenuation as a remedial alternative (Pennington et al. in preparation). To that end, project objectives were as follows:

a. To refine groundwater-monitoring procedures to optimize data quality so that observed trends in explosives concentrations over time are reliable.

b. To evaluate the significance of site-capacity measurements on the ultimate fate and transport of explosives at the demonstration site.

c. To investigate the application of microbial biomarker techniques for monitoring attenuation processes and rates.

d. To investigate the feasibility of using stable isotopes to monitor attenuation processes.

e. To apply conceptual and numerical models for contaminant definition and predictions of future contamination mobility and extent.

Rationale

Research in support of development of guidance for selection and implementation of natural attenuation for explosives was focused in the following areas: (a) groundwater monitoring, (b) site-capacity estimation parameters, (c) biomarkers, (d) stable isotopes, and (e) modeling. This report contains results for the demonstration site at Louisiana Army Ammunition Plant (LAAP) and selected results from the validation site. (For complete results at the validation site, see Pennington et al. (1998).) All results for stable isotopes will appear in a follow-on report.

Groundwater monitoring

Declining concentrations of explosives in groundwater over time may be evident from site historical data. If adequate, these historical records provide the first line of evidence under the EPA policy directive, "Historical groundwater and/or soil chemistry data that demonstrate ... declining contaminant mass and/or

concentrations...." The first task was to evaluate the extensive historical data at LAAP for trends in explosives concentration. Since long-term monitoring is required to verify any observed trends, development of a monitoring plan was the next task. To optimize the validity of trends, attention was focused on the quality of the data generated by the groundwater-monitoring plan. Special emphasis was placed on development of techniques for ensuring quality data. Efforts included techniques for minimizing the influence of oxygen at the well head on sampling formation water and maintaining sample integrity, sample preservation, precision, accuracy and representativeness of the data, data comparability, field quality control, and confirmation of analytical chemistry. For monitoring data to support the second line of evidence, "Hydrogeologic or geochemical data that can be used to indirectly demonstrate the type(s) [and rate] of natural attenuation processes active at the site...," collection of data that were not collected during previous monitoring was required. Therefore, an extensive list of explosives-transformation products and geochemical parameters was included in the analyses. The monitoring plan developed for the demonstration site, LAAP, was validated at a second site, Joliet Army Ammunition Plant (JAAP), Joliet, IL.

Site capacity

Site capacity for attenuation of explosives is a function of soil sorption, biodegradation, transformation, and chemical interactions with soil organic matter and clay. All of these processes are not well defined for explosives. Nonetheless, site capacity can be measured by simple batch shake and column tests that quantify contaminant half-life and adsorption coefficients. These capacity measurements can be used to refine predictive capabilities of numerical models for the site. Both batch shake and column tests were performed on soils from the LAAP aquifer to quantify sorption coefficients and disappearance rates for explosives. These data can support the second line of evidence by providing attenuation rate measurements.

Biomarkers

Biomarker techniques have been used to detect the involvement of microorganisms as biocatalysts for the transformation and degradation of contaminants. The techniques have been used extensively to measure changes in microbial community structure and microbial response to contaminants even after the contaminant is no longer detectable (e.g., the plume has migrated beyond the microbial community, or the contaminant concentration has been reduced to below detection).

Radiorespirometry provides direct evidence that transformation and complete mineralization can occur in site soils by challenging the indigenous microflora with radiolabeled TNT and RDX. Mineralization is evidenced by evolution of radiolabeled carbon dioxide. Analyses of bacterial polar lipids provide information on microbial community biomass and composition and the changes resulting from anthropogenic chemical perturbations. Analysis of nucleic acids provides a mechanism to detect the presence of genes encoding enzymes required for explosive degradation in situ. The radioassay results can be correlated with results from the

two biomarkers and site geochemical data to build a case for natural attenuation onsite. Biomarkers demonstrate (a) whether a viable biomass is present, active, and capable of metabolizing RDX and TNT, (b) the presence of catabolic genes necessary for in situ degradation, and (c) whether significant positive correlations to explosive concentrations, geochemistry, and mineralization properties exist.

The third line of evidence, "Data from field or microcosm studies ... which directly demonstrate the occurrence of a particular natural attenuation process... to degrade the contaminants of concern...," generally requires laboratory testing of field samples (groundwater and aquifer material). Therefore, samples of aquifer soils collected by cone penetrometry were subjected to biomarker analyses in laboratory microcosms. Soils from LAAP were used for development and refinement of techniques, while soils from JAAP were used to validate procedures. The two biomarker techniques, phospholipid fatty acids analysis and microbial DNA analysis, were coupled with radiorespirometry in microcosm studies.

Modeling

Modeling is essential for (a) conceptualization of the contamination at the site so that proximity to receptors can best be determined, (b) evaluation of factors dominating natural attenuation processes at the site, and (c) prediction of long-term contaminant migration and transformation. A numerical model was applied to the LAAP site using results from the other focus areas, i.e., groundwater monitoring, site-capacity determinations, and biomarkers, in addition to local weather data and other published information. A comprehensive computer graphical and integral modeling system, the Department of Defense Groundwater Modeling System (GMS) (1996) was used. The model contains tools to facilitate site characterization, conceptualization, geostatistical computations and postprocessing. The model links transport and water quality models to predict the fate and transport of contaminants. Sensitivity of the model simulations and predictions to input parameters was coupled with the desired level of accuracy to determine the level of detail required for field and laboratory measurements. Contaminant mass was also calculated using the measured and predicted explosives concentrations from the model.

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2 Groundwater Monitoring and Cone Penetrometry

Introduction

The primary site for demonstration of natural attenuation of explosives was Area P at LAAP, Minden, LA. This site was selected because the source of contamination had been removed, extensive historical contaminant and geological data were available, and more than 50 groundwater monitoring wells were in place. A small site at JAAP, Joliet, IL, was added to provide verification of the approaches developed at LAAP. Objectives of the monitoring were to determine trends in groundwater concentrations over time and provide data to support conceptual and predictive modeling for each site. Objectives of the cone penetrometry sampling were to refine conceptualization of site lithology and contaminant distribution in the subsurface and to provide site material for development of biomarkers as monitoring tools for natural attenuation of explosives.

Site Description and Historical Perspective: LAAP

Site location and description

The LAAP is a government-owned, contractor-operated facility located 22 miles[1] east of Shreveport, LA (Figure 1). The LAAP is bound to the north by Interstate 20 and U.S. Highway 80, to the south by State Route 164, to the east by Dorcheat Bayou, and to the west by Clarke Bayou. Two streams, Boone Creek and Caney Creek, flow north to south across the site. The facility lies within the Bossier and Webster parishes. The 14,974 acres within LAAP are predominantly woodlands (80 percent); approximately 20 percent of the area is occupied by former production lines and mission support facilities.

[1] A conversion table for converting non-SI units to SI units is provided on page vii.

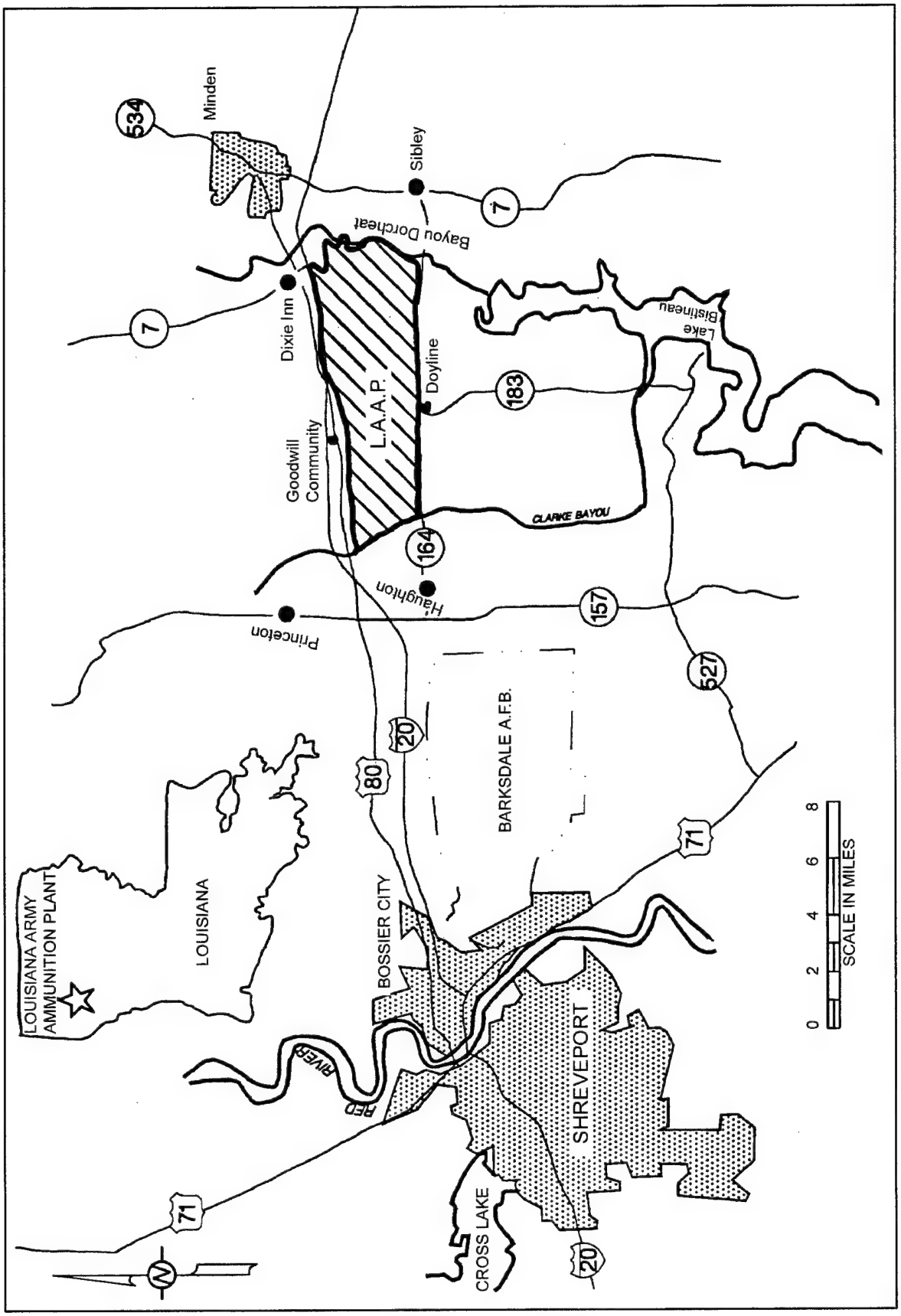

Figure 1. Geographical location of LAAP, Minden, LA (To convert miles to meters, multiply by 1.609)

Site function and evolution

The primary mission of the LAAP was to load, assemble, and package explosives into shell casings, manufacture ammunition metal parts, and provide associated support functions for ammunition production. Eight ammunition lines and one ammunition nitrate graining plant were constructed by the Silas Mason Company between July 1941 and May 1942. Production ceased in August 1945 at the conclusion of World War II. The plant was placed on standby status in September 1945, and contract operations were terminated in November 1945.

In February 1951, with the outbreak of the Korean Conflict, Remington Rand reactivated the plant under contractual agreement with the Federal government. Ammunition production was suspended in October 1957, and again the facility was placed on standby status. The Federal government again reactivated the facility in September 1962 and contracted with Sperry Rand Corporation to operate munitions production in support of the Vietnam Conflict. In 1974, Thiokol Corporation took over the facility operations when Sperry Rand Corporation relinquished its contract. Thiokol Corporation maintained the facility until the summer of 1996 when most operations at the plant ceased. As of August 1997, five contractors were bidding to resume very limited production of "black powder" at a single load line (Y line).

The LAAP was placed on the National Priorities List (NPL) in March 1989 because of contamination caused by past disposal of explosives-laden wastewater in 16 unlined surface impoundments located in Area P (Figure 2). An interim remedial action was initiated in 1988 because investigations indicated that the lagoons were contributing explosives to the groundwater. The lagoons were remediated by draining and treating wastewater and incinerating soils. The lagoons were excavated until a total field-determined explosive concentration of less than 100 mg explosives kg^{-1} soil was reached. The incineration of 101,929 tons of soils and the treatment of 53,604,490 gal of wastewater and rainwater collected within the 16 lagoons were completed in 1990. The area was then backfilled with the incinerated soil, capped, and vegetated. The 26-acre site was covered with a minimum 2-ft-thick compacted cap of uncontaminated clay soil from Area P and a nearby borrow pit located north of the lagoons. This clay cap covered all of the original Area P including the former lagoons and was compacted to at least 90 percent of the standard proctor density for the clay used. The cap was covered with 4 in. of topsoil with a slope of at least 1 percent to facilitate drainage.

The predominant contaminants at LAAP were TNT and RDX. Previous studies conducted in conjunction with remedial action on the lagoons had defined two subsurface geological terraces. A conceptualization of the contaminant plume and general groundwater hydrology and site geology had been made (Science Applications International Corporation 1994). Therefore, extensive historical contaminant and site-characterization data existed for Area P. These data provided a spring-board for development of the groundwater-monitoring plan.

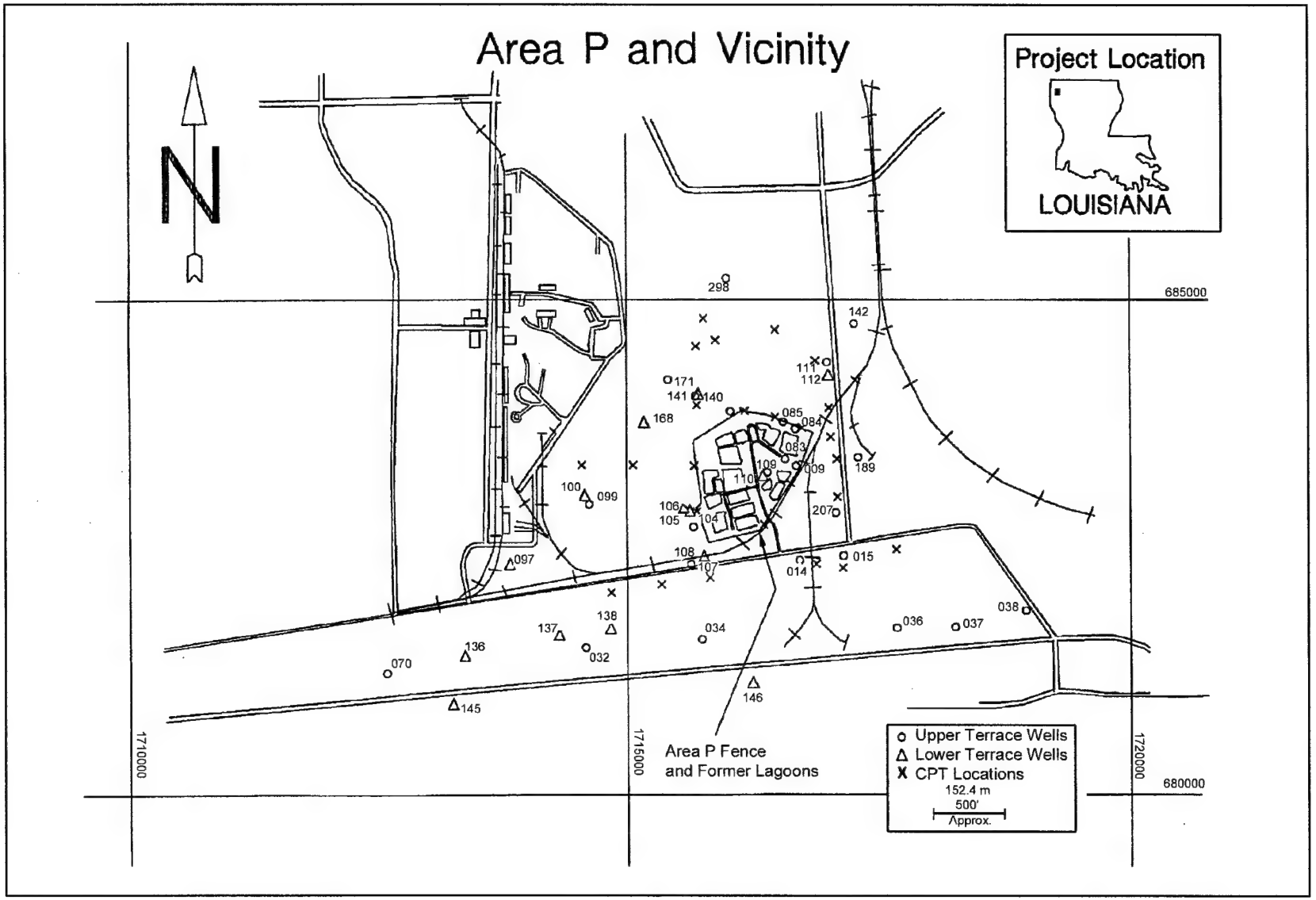

Figure 2. Area P at LAAP with former munitions disposal lagoons, fenced and capped remediation area, and monitoring-well locations

Physiography and geology

Physiography. The LAAP lies within the Western Gulf Coastal Plain physiographic province. Two major land forms, dissected uplands and rolling prairie, are found within LAAP. Minor land forms include abandoned channels, typically filled with clay that was deposited by ancient courses of the ancestral Red River. Relief at LAAP is moderate with elevations varying from about 130 ft above mean sea level (MSL) near Dorcheat Bayou to 80 ft above MSL at Clarke Bayou.

Regional geology. Regionally, LAAP lies within a subsurface structural feature known as the North Louisiana Syncline. This syncline lies on the eastern limb of the much larger Sabine Uplift. The uplift was formed by deformation of sediments during tectonic activity that began approximately 225 million years ago (Paleozoic Era). The North Louisiana Syncline and the LAAP region are bounded to the south and east by the Monroe Uplift and to the west and north by the Sabine Uplift. Smaller local uplifts exist in the area and can significantly modify the local structural geology (i.e., formation dip) and groundwater flow regimes.

Site geology. The surface at Area P consists of Pleistocene Age terrace deposits unconformably overlying the Eocene Age, Cane River Formation. Collectively, the Pleistocene Age units are a fining upwards sequence deposited in a fluvial-deltaic environment. The terrace deposits in Area P can be further subdivided into the Lower Terrace consisting of fine sands and a trace of gravel and the Upper Terrace consisting of very fine silts, clays, and silty clays. An intermediate clay unit is present at some locations, but is totally absent at many locations. Where present, this layer can serve as a limited aquitard. The Eocene Age, Cane River Formation directly underlies all terrace deposits and consists of clay or clay sufficiently indurated to be classified as claystone. The Cane River is not an aquifer beneath Area P, and is, therefore, considered the confining layer for modeling the site. Earlier geological descriptions of LAAP including Area P were reviewed prior to initiation of groundwater monitoring (Louisiana Department of Conservation 1954; U.S. Geological Survey 1983; U.S. Army Corps of Engineers 1984 and 1987; Engineering Technologies Associates 1991; Science Applications International Corporation 1994; International Technology 1997).

Hydrogeology. Groundwater in the Upper Terrace aquifer generally exists under water table (unconfined) conditions at depths varying from approximately 5 to 25 ft below ground level (BGL). The Lower Terrace aquifer, while not present in all areas, typically occurs from 25 ft BGL to the top of the Cane River, which is about 50 ft BGL. The Lower Terrace aquifer also tends to produce more water than the Upper Terrace deposits. Although none of the terrace deposits supply water to production wells on the installation, some domestic wells in Haughton Princeton, Dixie Inn, Minden, Sibley, and Doyline are completed in the terrace deposits. Groundwater quality modeling conducted for Area P indicated that contaminant (explosives) migration in the Upper Terrace generally traveled downwards with little horizontal spreading (Engineering Technologies Associates

1991). Furthermore, the modeling and water-level measurements indicated that the regional groundwater flow in the Upper Terrace aquifer was southwest.

Historical contaminant data

Science Applications International Corporation (SAIC) (1994) under contract to the U.S. Army Environmental Center (USAEC) conducted a 5-year review to assess the effectiveness of the interim remedial action at Area P. The review was conducted in accordance with the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) of 1980. The final report was submitted to USAEC in August of 1994. A statistical regression analysis approach was used to identify groundwater trends. Groundwater sampling data were evaluated from 1980 through 1994. Quadratic and linear analyses were conducted for 108 data sets. Trend categories were assigned to each of the data sets based on improving deteriorating and stable groundwater quality with regards to explosives. In these data sets, no specific trends were identified, but the general conclusion was that the overall quality of water in the Upper and Lower Terrace aquifers at Area P was improving.

Site Description and Historical Perspective: JAAP

Site location and description

The JAAP was constructed in the early 1940s in the Kankakee and Des Plaines river valleys in Will County, IL, about 17 miles south of Joliet, IL (Figure 3). The site encompasses approximately 44 square miles and is divided by U.S. Highway 53. The area to the west of Highway 53 covers approximately 14 square miles and functioned in munitions manufacture. The area to the east covers approximately 27 square miles and functioned in LAP of munitions. The study area, Site L1, consists of approximately 80 acres located in the north-central portion of the LAP area.

Site function and evolution

The JAAP is a government-owned, contractor-operated installation currently maintained in nonproducing status. The JAAP was used extensively during World War II. In August of 1945, production of explosives halted, the sulfuric acid and ammonium nitrate plants were leased, and remaining productions facilities were placed in standby status. The explosives manufacturing area was reactivated during the Korean Conflict (1953 to 1957) and again during the Vietnam Conflict (1965 to 1969). Production gradually decreased until it was stopped in 1977. Currently, various tracts of land in the 30,000-acre site are transitioning to the Department of Interior as part of the National Tall Grasslands program. Additional tracts are leased for agriculture. During the Installation Assessment and Installation Restoration Surveys, site conditions suggested the potential for contamination from past operations (Donohue and Associates 1982a,b).

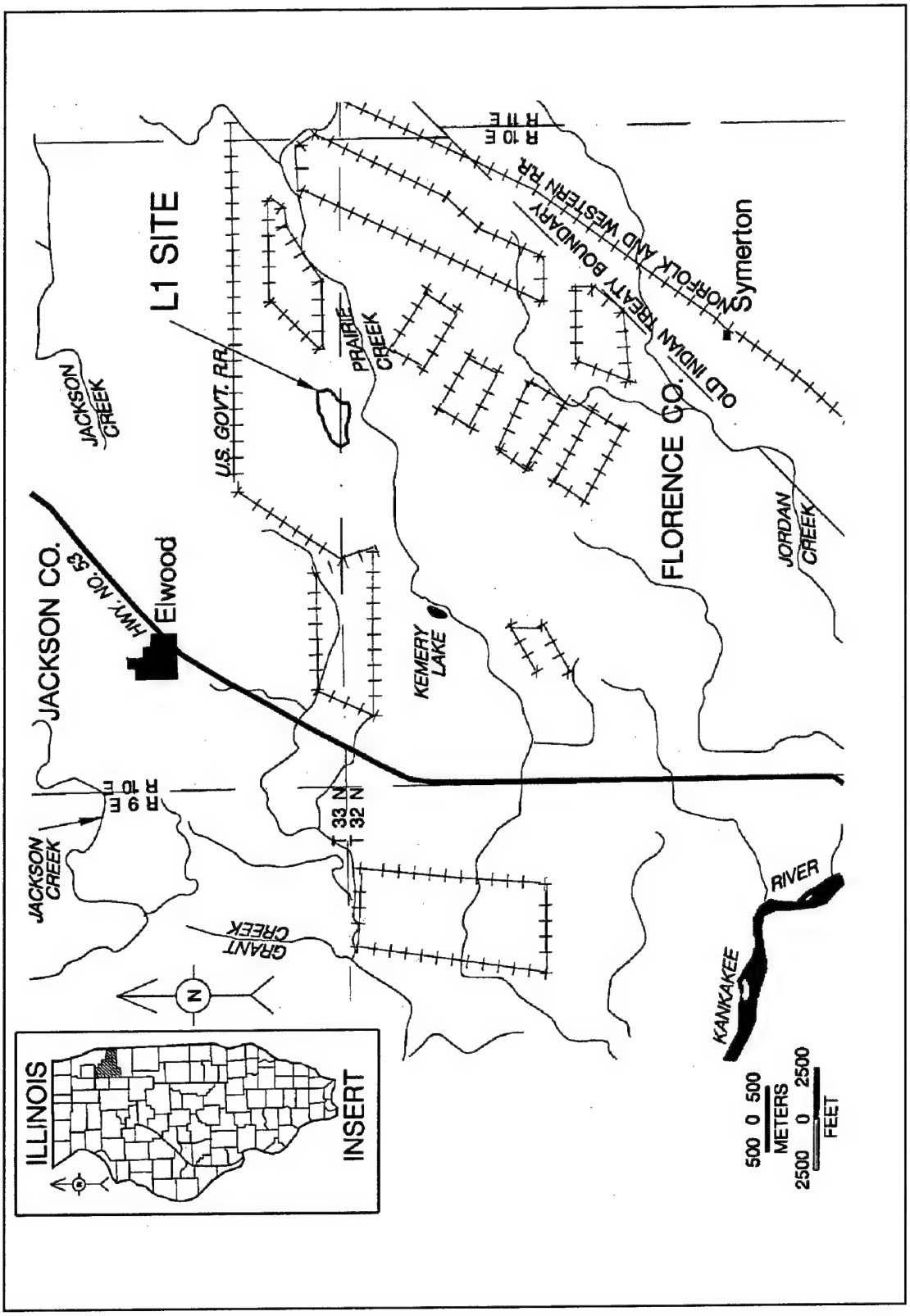

Figure 3. Geographical location of JAAP, Joliet, IL, with Site L1

Subsequent studies identified contamination in the groundwater, surface water, soil and sediment at the LAP areas (Dames and Moore, Inc. 1993). The LAP area was placed on the NPL in April 1989, and the site was designated a Superfund site. A Remedial Investigation (RI) identified 35 specific study sites in the LAP area as potentially contaminated (Dames and Moore, Inc. 1993).

Site L1 functioned from 1941 to 1945 in defusing of munitions, removal of explosives from shells and recycling of casings, crystallization of ammonium nitrate, and TNT recovery. From 1946 through 1952, the site functioned in reclamation of TNT from shells (Dames and Moore, Inc. 1996). The principal source of explosives contamination at Site L1 is a ridge and furrow system (10 acres) that received process wastewater from washout operations (Dames and Moore, Inc. 1993). Extensive additional site history is given in the Feasibility Study, Ground Water Operable Unit (Dames and Moore, Inc. 1996) and in the Phase 1 Remedial Investigation Results Report, Load-Assemble-Package (LAP) Area (Dames and Moore, Inc. 1993).

Prior remedial actions. Between 1994 and 1995, Argonne National Laboratory conducted a field demonstration of slurry reactor biotreatment of explosives-contaminated soils at Site L1 (Manning, Boopathy, and Breyfogle 1996). Soils for the demonstration were removed from the ridge and furrow system. After the demonstration, treated soils were left on the site. Between 1993 and 1995, a plant uptake study was conducted at Site L1 (Zellmer et al. 1995). The study consisted of a plant and soil survey for explosives and a cropping experiment using two plant species grown in soils amended with three levels of chopped grass hay. The cropping experiment was established on a 16- by 24-m plot in the ridge and furrow system (high-TNT area) and a plot of the same size west of the ridge and furrow system (intermediate-TNT area). Results indicated no TNT nor transformation products in aboveground plant tissues of existing or cropped vegetation, but low concentrations associated with the roots. Crop health was positively related to amendment level.

Prior trend analysis. Prior to initiation of monitoring, historical contaminant concentration data taken from groundwater wells in 1981, 1983, 1985, 1986, and 1991 (Dames and Moore, Inc. 1993) were analyzed for the following explosives and derivatives: 2,4DNT; 2,6DNT; DNB; octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX); RDX; tetryl; TNB; and TNT. Data sets were relatively complete for 1983, 1985, 1986, and 1991 for seven wells and the analytes TNB, TNT, 2,4DNT, and 2,6DNT (Table 1). Observations of the data showed that concentrations were decreasing in about 45 percent of the data sets for which concentrations exceeded detection limits (6 of 13 values). Analytes decreasing in concentrations were TNB (one data set), DNB (one data set), TNT (two data sets), 2,4DNT (one data set), and tetryl (one data set). Since these observations were spotty, the data were insufficient to define statistical trends in contaminant concentrations over time.

Table 1
Historical Groundwater Concentrations (μg L^{-1}) of Explosives and Degradation Products for Site L1, JAAP[1]

Well No.	Date	TNB	DNB	TNT	2,4DNT	2,6DNT	HMX	RDX	Tetryl
MW131	06/10/81	1,291.00[2]	NT[3]	2,250.000[2]	1.29[2]	3.75[2]	NT	NT	NT
	1983	NT	NT	NT	NT	NT	NT	NT	NT
	11/15/85	1,610.000	5.000	2,150.000	2.010	4.140	NT	7.00 DL[4]	58.600
	04/22/86	755.000	2.300 DL	576.000	0.560 DL	8.540	NT	7.00 DL	21.700
	08/21/91	1,300.000	0.611 DL	1,900.000	0.064 DL	0.074 DL	1.210 DL	38.600	2.490 DL
MW172	03/09/83	9.200	NT	40.800	0.280 DL	3.00 DL	NT	NT	NT
	09/28/83	2.800 DL	NT	10.600	0.280 DL	3.000 DL	NT	NT	NT
	10/30/85	3.080	2.300 DL	16.200	0.560 DL	1.200 DL	NT	14.200	5.600 DL
	10/14/86	3.840	2.300 DL	12.900	0.560 DL	1.200 DL	NT	7.220	5.600 DL
	08/23/91	0.449 DL	0.611 DL	2.340	0.064 DL	0.074 DL	1.210 DL	8.790	2.490 DL
MW173	03/09/83	6.870	NT	50.300	0.280 DL	3.000 DL	NT	NT	NT
	09/28/83	2.800 DL	NT	68.400	0.280 DL	3.000 DL	NT	NT	NT
	10/31/85	14.00	2.30 DL	105.00	0.560 DL	1.200 DL	NT	56.500	5.600 DL
	04/14/86	2.090	2.300 DL	11.00	0.560 DL	1.200 DL	NT	8.000	5.600 DL
	08/23/91	5.308	0.611 DL	55.000	0.064 DL	0.074 DL	43.800	42.100	2.490 DL
MW174	03/09/83	2.800 DL	NT	0.310 DL	0.280 DL	3.000 DL	NT	NT	NT
	09/28/83	2.800 DL	NT	0.610	0.280 DL	3.000 DL	NT	NT	NT
	10/31/85	1.400 DL	2.300 DL	1.900 DL	0.560 DL	1.200 DL	NT	7.000 DL	5.600 DL
	04/14/86	1.400 DL	2.300 DL	1.900 DL	0.560 DL	1.200 DL	NT	7.000 DL	5.600 DL
	08/23/91	0.449 DL	0.611 DL	0.635 DL	0.064 DL	0.074 DL	1.210 DL	1.170 DL	2.490 DL
MW175	03/10/83	2.800 DL	NT	0.310 DL	0.280 DL	3.000 DL	NT	NT	NT
	04/14/86	1.400 DL	2.300 DL	1.900 DL	0.560 DL	1.200 DL	NT	7.000 DL	5.600 DL
	11/13/91	0.449 DL	0.611 DL	0.635 DL	0.064 DL	0.074 DL	1.210 DL	1.170 DL	2.490 DL
MW177	03/09/83	2.800 DL	NT	0.310 DL	0.280 DL	3.000 DL	NT	NT	NT
	09/28/83	2.800 DL	NT	0.310 DL	0.280 DL	3.000 DL	NT	NT	NT
	10/30/85	1.400 DL	2.300 DL	1.900 DL	0.560 DL	1.200 DL	NT	7.000 DL	5.600 DL
	04/14/86	1.400 DL	2.300 DL	1.900 DL	0.560 DL	1.200 DL	NT	7.000 DL	5.600 DL
	08/23/91	0.449 DL	0.611 DL	0.635 DL	0.064 DL	0.074 DL	1.210 DL	1.170 DL	2.490 DL
MW178	03/09/83	2.800 DL	NT	0.380	0.280 DL	3.000 DL	NT	NT	NT

(Continued)

1 Dames and Moore, Inc. (1993).
2 Mean of two samples collected in duplicate.
3 Not tested.
4 Detection limit.

Table 1 (Concluded)

Well No.	Date	TNB	DNB	TNT	2,4DNT	2,6DNT	HMX	RDX	Tetryl
MW178	11/06/85	1.400 DL	2.300 DL	1.900 DL	0.560 DL	1.200 DL	NT	7.000 DL	5.600 DL
	04/14/86	1.400 DL	2.300 DL	1.900 DL	0.560 DL	1.200 DL	NT	7.000 DL	5.600 DL
	08/21/91	0.449 DL	0.611 DL	0.635 DL	0.064 DL	0.074 DL	1.210 DL	1.170 DL	2.490 DL

Physiography and geology

Physiography. JAAP is located within the northern portion of the Central Lowlands physiographic province. This province is characterized by relatively flat topography and low relief. The most prominent topographic feature of JAAP is a 50-ft-high glacial escarpment that trends north-south across the installation. The installation is drained by four streams: Grant Creek, Prairie Creek, Jordan Creek, and Spoil Bank Creek.

Regional geology. Regionally, JAAP is located on a large structural high called the Kankakee Arch. This feature is located between the Michigan Basin to the northeast and the Illinois Basin to the south. Faulting in the area includes the Sandwich Fault Zone, which trends northwest-southeast through the eastern portion of JAAP. Structural contour maps indicate that vertical displacement of the fault increases with depth and that the fault may affect groundwater flow locally (Kolata, Bushbash, and Treworgy 1978; U.S. Army Environmental Hygiene Agency 1977). However, no regional effects have been observed (Viscocky 1985; Suter et al. 1959).

Local geology. Site L1 has been contaminated by the use of a 10-acre ridge and furrow system, i.e., an evaporating bed, that received process wastewater from washout operations (Figure 4). Ground-surface elevations at Site L1 vary from 650 ft above MSL along the northern border to approximately 610 ft above MSL along Prairie Creek to the south. Surface runoff flows into three drainage ditches that ultimately flow into Prairie Creek. Prairie Creek in the vicinity of Site L1 is 10 to 15 ft wide and flows from east to west.

The soils at Site L1 are glacial materials composed of very fine-grained silts and clays with various amounts of erratically occurring materials such as cobbles and boulders. These materials were deposited as outwash during the waning stages of continental glaciation. The glacial materials lie unconformably on bedrock that consists of tan to greenish-gray thinly bedded Silurian-age dolomitic sandstone. The sandstone is highly fractured and weathered near its contact with the overlying glacial deposits and has solution features in the upper portion of the section. In general, the fractures and degree of weathering in the sandstone decrease with depth. Hydraulic conductivities are generally very low. A hydraulic conductivity of 9.2×10^{-6} cm sec^{-1} was reported for MW131 (Donohue and Associates 1982b), which is completed in the glacial till (described as overburden in Dames and Moore, Inc. 1994). Hydraulic conductivities for these materials are

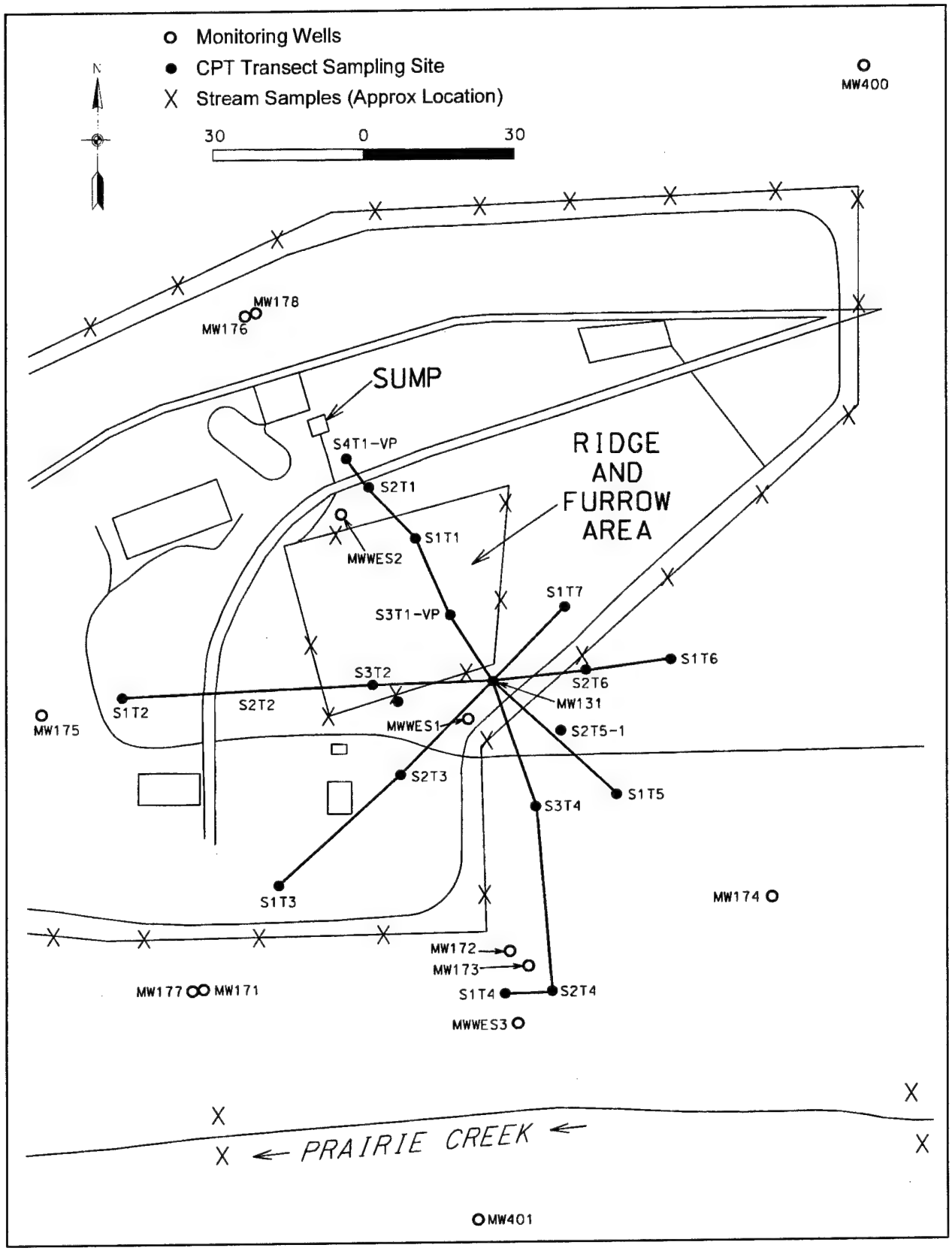

Figure 4. Locations of monitoring well and cone penetrometry sampling points at Site L1, JAAP

typically low, while conductivities in the bedrock are much higher. The high bedrock conductivities are attributed to solution features in the upper portions of the bedrock. Using slug test data, a hydraulic conductivity of 4.9×10^{4} cm sec^{-1} was calculated for the bedrock (Dames and Moore, Inc. 1994). Based on these conductivity values, groundwater flow velocities were estimated at 35 and 16 ft $year^{-1}$ for the bedrock and glacial till, respectively. The approximate direction of groundwater movement is to the southwest in the deep wells and southeast in the shallow wells (Dames and Moore, Inc. 1994).

Historical contaminant data

Between 1986 and 1991, groundwater data were collected five times in Site L1 (Dames and Moore, Inc. 1993). The following six explosives were detected in exceedance of preliminary remediation goals (PRGs): TNT, TNB, RDX, 2,4-dinitrotoluene (2,4DNT), 2,6-dinitrotoluene (2,6DNT), and 1,3-dinitrobenzene (DNB) (Table 1). All of the PRG exceedances were in wells MW131, MW172, and MW173 (Dames and Moore, Inc. 1993). Detections of 2,4DNT and 2,6DNT were low; maximum detections were 2.01 and 8.54 $\mu g\ L^{-1}$, respectively.

Materials and Methods: LAAP

Analysis of historical data

Data limitations. Historical data were obtained from the USAEC for 66 wells in or near Area P for the time period 1980-1995. These included 61 monitoring wells, two water wells just west of Area P, and three water wells just south of Area P near the town of Doyline. All concentration data for nine explosives were used in the historical trend analysis except the following:

- Data prior to 1986. These data were considered to have poor precision relative to later data; methods descriptions for pre-1986 chemical analyses were not available.[1]

- Less than detection limit data with detection limits $\geq 4.9\ \mu g\ L^{-1}$. Most detection limits were $<2\ \mu g\ L^{-1}$, but in some cases detection limits were as high as $100\ \mu g\ L^{-1}$. Most detection limits $\geq 4.9\ \mu g\ L^{-1}$ resulted from dilutions[1] and were considered too imprecise to be useful in the data analysis. Since the range in detections spanned several orders of magnitude dwarfing detection limit values, all remaining less than detection limit data were set equal to the detection limit for statistical analysis.

[1] Personal Communication, April 1998, Mr. Doug Scarborough, U.S. Army Environmental Center, Aberdeen Proving Ground, MD.

- Data from the water wells, which were consistently below detection limit, were used in mapping contaminant concentrations, but were not included in statistical analyses.

Data were analyzed separately for the Upper Terrace aquifer (32 wells) and the Lower Terrace aquifer (29 wells). To minimize seasonal influences, mean yearly concentrations for each explosive from each well were used.

Time series contaminant data were sparse. Most wells were sampled during only 1 or 2 years of the 1986-1995 time period. No wells were sampled in 1992; in 1987, 1991, and 1993, only water wells were sampled. The most extensive data set was available for 1990, during which 62 wells were sampled. Because of the substantial data gaps in the time series, grouping of wells, rather than evaluation of individual wells, was necessary to provide enough data for meaningful analyses.

Contaminant concentration mapping. For each explosive and each terrace, well data were plotted on maps of Area P using the following concentration categories:

a. Less than detection limit whenever sampled.

b. Greater than detection limit at some point during the time period (1986-1995), but always <10 $\mu g\ L^{-1}$.

c. Greater than 10 $\mu g\ L^{-1}$ at some point during the time period.

d. Greater than 100 $\mu g\ L^{-1}$ whenever sampled.

Wells in categories *c* and *d* were used to draw boundaries for a more highly contaminated Inner Zone, while wells in category *b* were used to draw boundaries defining a less contaminated Outer Zone. Wells in category *a* outside of the Outer Zone were excluded from subsequent analyses because such data would only contribute to trends in detection limits. The Inner and Outer zones were analyzed separately to facilitate trend detection.

Trend analysis. The objective of the trend analysis was to determine whether groundwater concentrations of any explosive in either zone in either terrace were significantly increasing or decreasing over time during the period 1986-1995. The trend analysis consisted of the following four parts:

a. Normality testing. Ordinary least-squares regression analysis assumes that the data are normally distributed. Gross violation of this assumption can result in inflated Type I error rate (i.e., saying that a significant trend exists when in reality there is no trend). For each explosive, terrace, and zone, the normality of untransformed and log-transformed concentration data was tested using Shapiro-Wilk's Test. Residuals (difference between each observation and the yearly mean of all wells in the group) were used in the normality test. Normality was rejected if Shapiro-Wilk's W statistic

was <0.90. For most explosives, log transformation of the data improved normality.

b. *Regression analysis*. Least-squares regressions of contaminant concentration on year were performed for each explosive, terrace, and zone, in most cases using log-transformed data. Both linear and quadratic models were tried. A significant regression F statistic ($P < 0.05$) indicated increasing trend in concentration over time when the slope was positive, and decreasing when the slope was negative, in the latter part of the time period.

c. *Lack-of-fit analysis*. Lack-of-fit analysis is a statistical test of how well a particular regression model fits the observed data. Lack-of-fit analysis can be conducted when one or more values of the criterion (Y) variable (i.e., sample year) have replicate observations of the predictor (X) variable (i.e., contaminant concentration). A regression model can be significant but still have poor fit to the data. When linear fit is poor, a low-order polynomial model such as quadratic regression will often improve the fit. Lack-of-fit analysis was performed for each regression model. Fit was rejected if P was less than 0.05 ($P < 0.05$) for the lack-of-fit F statistic, regardless of whether the regression slope was significant.

d. *Rank correlation analysis*. If normality could not be satisfied using either untransformed or log-transformed data, then a nonparametric test for trend was performed by calculating Spearman's rank correlation statistic (ρ) between contaminant concentrations and sample year. A significant positive ρ ($P < 0.05$) indicated increasing trend in concentration over time; a significant negative ρ ($P < 0.05$) indicated decreasing trend in concentration over time.

Well selection

Sixty-one wells were located in and around Area P at LAAP at the inception of the demonstration project. Some of these wells were rejected in the first field reconnaissance because well diameters were too small to accommodate the 5-cm pump adopted for groundwater sampling. Others had been sealed for various reasons or provided insufficient flow rates for sampling.

Historical data from the wells in the Area P vicinity were reviewed to find any trends of declining concentration of TNT, RDX, TNB, or 2,4DNT. The objective was to select wells from both the Upper and Lower terraces. Selection criteria included spatial distribution of wells relative to the original source and relative to the existing conceptualization of the contaminant plume (Dames and Moore, Inc. 1993), location of potential receptors, and availability of well completion data concerning screen type, depth, and well performance, such as water yields. Thirty wells were selected (Figure 2). During the first 6 months, monitoring was scheduled monthly in order to refine the sampling protocol, sampling techniques, and sample-handling procedures. Analyses of these data were also used to indicate the variability that could be expected in contaminant and geochemical data

from the site. After the first 6 months, sampling was reduced to quarterly for the duration of the 2-year demonstration period. After the first year, the number of wells were limited to 16 to reduce the expense of repeated monitoring of wells that had been consistently uncontaminated and were not within the immediate vicinity of the contaminant plume. All 30 wells were sampled again at the end of the second year.

Data quality assurance

Data quality objectives. One intent of the well sampling was to provide a 2-year data set to assess whether concentrations of explosives in the groundwater at LAAP are decreasing with time because of natural processes within the aquifer. An important objective was to minimize contributions of random error and systematic bias to determine whether observed trends in analyte concentrations were statistically significant. The following tests were executed to minimize data artifacts.

Stabilization of explosives concentrations. Initial experiments were conducted during the first month of sample collection to optimize standard operational procedures for sample collection during the 2-year monitoring program. The first experiment was designed to determine the relationship between well-purging time and concentrations of explosives and their transformation products. Between well-sampling events, the groundwater in the immediate vicinity of the well head has the opportunity to become oxidized because of contact with the air column in the well. The extent of the oxygenated zone around the well head is a function of local permeability of the aquifer, the flow rate of the groundwater, and the time since last well purging. Conditions in this zone may significantly affect the characteristics and chemistry of the contaminant and introduce artifacts into the contaminant data. Therefore, samples from three wells (MW168L, MW100L, and MW110L) were collected every 10 min until the dissolved oxygen readings were stable (varied at each well, but generally less than 2 hr). Samples were analyzed for TNT, RDX, 4ADNT, 2ADNT, 2,4DNT, 3,5DNA, and TNB (see methods below). Dissolved oxygen readings were recorded each time a sample was taken. Pearson Product Moment Correlations between explosives concentrations, dissolved oxygen, and temperature were examined.

Sample preservation. A second experiment assessed whether sample preservation was essential to prevent analyte loss during the period between sample collection and analysis. Samples were collected from 10 wells. Each sample was split into three aliquots: one aliquot served as a control and was not chemically preserved; a second aliquot was acidified to pH 2 with 1.5 g sodium bisulfate ($NaHSO_4$) L^{-1}; and the third aliquot was preserved by addition of 60 mg mercuric chloride ($HgCl_2$) L^{-1}. Samples were stored on ice and analyzed for explosives as described below. The statistical model used to compare treatment results was a two-way analysis of variance with preservative as the treatment variable and monitoring well as a blocking variable. Data were omitted from the analysis when (a) all or nearly all observations for an analyte were below detection limit, or (b) all observations for an analyte within a well were below detection limit or a

combination of nondetections and J values (quantifiable values that fell below the statistically significant detection limits).

Precision, accuracy, and representativeness. Precision was estimated from the agreement among replicate measurements. One of every ten samples was collected and analyzed in duplicate. Precision estimated from these samples included contributions from the entire measurement process including collection, storage, preconcentration, and determination. Precision was determined by a paired t-test.

Accuracy is a measure of the agreement of the determined value to the "true" value. Since the "true" value is unknown for any real sample, accuracy must be estimated from fortified (spiked) samples subjected to the entire analytical process. Two spiked blanks and three spiked groundwater samples were analyzed for each sampling event. Spiked blanks and spikes into low-concentration samples were made so that spiked concentrations were 1.0 $\mu g\ L^{-1}$. Spikes into high-concentration samples were made so that concentrations were 250 $\mu g\ L^{-1}$. Pooled means and standard errors were obtained by combining the results from 30 duplicate measurements for each of six analytes (TNT, RDX, HMX, TNB, 4ADNT, 2,4DANT). Pooled means were compared with 100-percent recoveries. Blank samples (unspiked reversed osmosis (RO) water) were also analyzed to ensure that no positive interference occurred for any of the methods.

Representativeness may be defined as the degree to which the data portrays the quality of the water in the aquifer with respect to time and location. To ensure temporal representativeness, samples were collected monthly for 6 months and then quarterly for 2 years. This allowed analysis of seasonal trends in the data. To detect differences because of location, 30 spatially distributed wells were sampled, including wells within the upper and lower terraces.

Comparability. Comparability was emphasized to minimize variability in contaminant concentration data caused by sampling and analysis artifacts. This was achieved by following the sampling protocol consistently for each well in each sampling event, adequately purging the well of oxygen influence in the well head before sampling, decontaminating the pump between wells, and randomly checking for contaminants in the rinsate at least once in each sampling round. Comparability was stressed during field sampling and chemical analyses to optimize detection of differences in explosives concentrations as a function of time.

Field quality control. Since concentrations of explosives ranged from less than the analytical detection limits (0.2 $\mu g\ L^{-1}$) to more than 20 $mg\ L^{-1}$, or about 5 orders of magnitude, the potential for carry-over of explosives as sampling progressed from well to well from high to low concentration was great. Therefore, wells were sampled in order from lowest to highest concentrations. To further minimize the opportunity for carry-over, Teflon tubing was dedicated to each well. The sampling pump was decontaminated between wells by placing it into a barrel of clean water and pumping for 10 min prior to placement into the next well. One

sample of rinsate from equipment decontamination was collected each sampling day for explosives analysis.

Split-sample analyses. One of every ten samples was split and analyzed independently using Method 8330 at the U.S. Army Cold Regions Research and Engineering Laboratory (CRREL) and by the U.S. Army Engineer Waterways Experiment Station (WES) Environmental Chemistry Branch. CRREL was the developer of Method 8330 (EPA 1994) and was well acquainted with its use for water analysis. Analytes included TNT, RDX, TNB, 4ADNT, 2ADNT, 2,4DNT, and HMX.

Groundwater monitoring methods

Sampling. The 30 wells were sampled by micropurge (low-flow) techniques (Gass et al. 1991). A 5-cm-diam low-flow pump was used for sampling wells having a diameter as small as 10 cm. Field parameters were measured with an in-line continuously monitoring unit (Yellow Springs Instruments, Yellow Springs, MO) with data transmitted directly to a laptop computer. Ecowatch software (Yellow Springs Instruments, Yellow Springs, MO) was used to visualize the parameters in real time. These parameters included pH, conductivity, dissolved oxygen (DO), temperature, redox, turbidity, and salinity. Field data were recorded with time. Discharge was matched to recharge using the low-flow pump until a stable DO reading was obtained. Initially, sampling procedures defined stability as achievement of replicate DO reading within 10 percent of each other. Once this was achieved, the groundwater was sampled. After two sampling events in which the time required to achieve a stable DO was consistent for each well, a minimum pumping time was established. On subsequent sampling rounds, the DO was monitored for the minimum pumping time to confirm this correlation between time and DO stabilization. An exception to this protocol was adopted for MW109U because recharge was too slow to use the micropurge technique. This well was evacuated and allowed to recharge for 10 min three consecutive times and then sampled.

Groundwater was pumped through the monitoring unit and discharged via Teflon tubing until time for sampling. Before sampling, the water flow was redirected through a 0.45-μm filter. The whole sample was collected in a 4-L brown glass bottle that had been certified precleaned. After thorough mixing, the sample was divided into separate bottles already containing appropriate preservatives for the specific analyses (see analytical chemistry below). The collection bottle was rinsed three times with deionized water between wells. Three subsamples of groundwater were distributed as follows: a 1-L sample for explosives, a 500-mL sample for nitrate/nitrite, total organic carbon, total iron, calcium, magnesium and manganese, and a 100-mL sample for sulfate and chloride. During Month 3 (Round 3, April 1996), a sample from each well was assayed for picric acid. Iron speciation, Fe^{+2} and Fe^{+3}, and methane were assayed in Month 10 (Round 7, Nov 1996) and in Month 12 (Round 24, February 1998) in selected wells (MW012U, MW014U, MW085U, MW099U, MW100L, MW104U, MW107L, MW108U, MW109U, MW110L, MW140U, and MW141L). Samples

for iron speciation and methane were collected by bailing after samples for explosives and other geochemical parameters had been collected by pumping. Samples were transferred from the bottom of the bailer into volatile organic analysis (VOA) tubes with silicon Teflon-faced septa using a volatile organic contaminants (VOC) removal device to reduce contact between the sample and air. All samples were stored on ice or under refrigeration until analyzed and were transferred under documented chain of custody. All monitoring wells, physical boundaries and features, and subsequent cone penetrometry (CPT) and surface soil-sampling locations were surveyed using a global positioning system.

Analytical chemistry methods: Geochemical parameters. Laboratory analyses included total iron, calcium, magnesium, and manganese (Method 6010, EPA 1988), total organic carbon (Method 505C, American Public Health Association 1985), nitrate-nitrite nitrogen (Method 353.2, EPA 1982), sulfate (Method 375.2, EPA 1982), and chloride (Method 325.2, EPA 1979). Samples for total iron, calcium, magnesium, and manganese, total organic carbon, and nitrate-nitrite nitrogen were preserved with 0.4 g $NaHSO_4$ to 250 mL of water. Samples for sulfate and chloride were not preserved. Iron speciation was achieved by ion chromatographic separation (Dionex Corp., Sunnyvale, CA) of samples preserved with 1 percent HCl followed by analysis according to Method 6020 (EPA 1988) on a Perkin Elmer (Norwalk, CT) inductively coupled plasma mass spectrometer.

For methane analyses, 20-mL aliquots of well water were transferred by gas-tight syringe to 40-mL VOA tubes. The samples were allowed to equilibrate with the headspace. A standard curve was developed by adding four concentrations of standard (100-percent pure) nitrogen gas to similar 40-mL VOA tubes containing 20 mL of distilled water. Headspace of standards and samples were quantified on a Model 8610 gas chromatograph (SRI Instruments, Las Vegas, NE).

Analytical chemistry: Explosives analyses. Historical data for wells sampled at Area P indicated that the most significant explosives on the site were TNT, TNB, and RDX (SAIC 1994). Other detections included HMX, tetryl, 2,6-dinitrotoluene (2,6DNT), 2,4-dinitrotoluene (2,4DNT), 1,3-dinitrobenzene (DNB), and nitrobenzene (NB). However, the presence of additional transformation products of TNT provides evidence for initial subsurface processes that may prove relevant to natural attenuation mechanisms. Therefore, the list of explosives analytes was expanded.

The target analytes for EPA SW846 Method 8330 (EPA 1994) include the following: HMX, RDX, TNB , DNB , tetryl, TNT, NB, 4-amino-2,6-dinitrotoluene (4ADNT), 2-amino-4,6-dinitrotoluene (2ADNT), 2,4-DNT, 2,6-DNT, o-nitrotoluene (2NT), m-nitrotoluene (3NT), and p-nitrotoluene (4NT). All of these analytes except for the mononitrotoluenes (2NT, 3NT, and 4NT) were assayed. In addition to these analytes, several other compounds have been identified as potential environmental transformation products of TNT, RDX, and TNB. Those from TNT and TNB include 3,5-dinitroanaline (DNA), 2,4-diamino-6-nitrotoluene (2,4DANT), and 2,6-diamino-4-nitrotoluene (2,6DANT) and three

isomeric azoxy compounds. All of these analytes were assayed except for the 2,2',4,4'-tetranitro-6,6'-azoxytoluene (66'AZOXY). Standards were available for unresolved 2,2',6,6'-tetranitro-4,4'-azoxytoluene (44'AZOXY) and 4,4',6,6'-tetranitro-2,2'-azoxytoluene (22'AZOXY) during the first 2 months of sampling; the resolved isomers, 22'AZOXY and the 44'AZOXY, were each available for samples from Months 3 through 5; and only the 44'AZOXY isomer was available for Months 6 through 12.

Samples were analyzed with and without a preconcentration step to broaden the range of detection from very low $\mu g\ L^{-1}$ to high $mg\ L^{-1}$ concentrations. Preconcentration was achieved by solid-phase extraction. The high performance liquid chromatography (HPLC) (Waters Corporation, Milford, MA) used an LC-18 reverse-phase (RP) column and an LC-CN RP confirmatory column having a slightly different retention time. Elution was with methanol/water (50/50 v/v). Analyte detection in the HPLC was achieved with an ultraviolet (UV) detector (EPA 1994).

Three nitroso derivatives of RDX have been observed as microbial transformation products (McCormick, Cornell, and Kaplan 1981). They are hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). All three of these analytes were assayed. Standards for MNX and TNX were available after Round 6; the standard for DNX was available after Round 8. Most of these compounds cannot be determined using Method 8330 as written. Therefore, a gradient elution RP-HPLC method was used. This method was designed to allow determination of compounds much more polar and much less polar than those on the Method 8330 target list. The standard for the mononitroso transformation product of HMX (MN-HMX) was also available after Round 11.

Some explosives-contaminated sites contain picric acid (most likely in the form of the picrate ion under environmental conditions), which was used during World War II in armor-piercing shells, bombs, and rocket warheads. During one round of sampling (Round 1, May 1997), visual observation of water (a fluorescent yellow-green coloration) from MW171U suggested the presence of picric acid. Analytical results indicated the presence of picric acid; therefore, groundwater from all other wells was analyzed for picric acid in Round 3, July 1997. Sample preparation for picric acid analysis was the same as for Method 8330. However, HPLC analysis used a mobile phase consisting of 40-percent methanol and 60-percent 0.5 M KH_2PO_4 buffer (pH adjusted to 3.5 with concentrated acetic acid). Elution was at 1.5 $mL\ min^{-1}$ for 10 min. The Waters 586 Tunable Absorbance Detector (Waters Corp., Milford, MA) was set at 363 nm, which is maximum absorbance for picric acid.

Statistical analyses: Trends in explosives concentrations. Trends in contaminant concentrations over the 2-year study period were analyzed statistically for the 11 wells in which most analytes were consistently detected. These included the upper terrace wells MW083U, MW085U, MW104U, MW109U, and MW140U and the lower terrace wells MW100L, MW105L, MW110L,

MW141L, and MW168L. Trend analysis was not attempted unless the data for a given well included at least three detectable concentrations. Explosives and derivatives that had at least three detectable concentrations in most or all of the 11 wells included 2,4DNT, 2ADNT, DNA, 4ADNT, DNB, HMX, RDX, TNB, and TNT.

Trend analysis was performed using the SAS LIFEREG procedure (SAS Institute, Inc. 1988) assuming either a normal or a lognormal distribution. The LIFEREG procedure fits a regression line by maximum likelihood estimation (MLE) and is particularly well suited to analysis of censored data such as those with below detection limit observations. LIFEREG incorporates probabilities below detection limit for the assumed distribution and does not require prior reconstitution of the censored data (e.g., using one-half the detection limit). However, a simulation study in progress has shown the LIFEREG procedure to have a high Type I (false positive) error rate in some circumstances. Therefore, trend analysis was also performed by ordinary least squares (OLS) regression using linear, logarithmic, and quadratic models in the SAS REG procedure, following substitution of one-half detection limit for less than detection limit observations. Trends were not considered valid unless significant ($P < 0.05$) by at least one of the MLE models and one of the OLS models. As a check on the validity of the regression trend analyses, nonparametric Spearman's rank correlations were performed, correlating contaminant concentrations with sample time in each well.

Statistical analyses: Correlation between geochemical parameters and explosives concentrations. Contaminant concentration data were correlated with concurrent geochemical parameters (Ca, Cl, Fe, Mg, Mn, NO_2/NO_3, SO_4, TOC, and water level) when the data for a given well included at least three detectable concentrations of both the contaminant and the geochemical parameter. Both Pearson's *r* and Spearman's nonparametric correlation analyses were conducted because so many explosives values were less than the detection limit and because sample size was relatively small ($n = 5$ to 12). Correlations were considered significant only when both analyses produced significant results ($P < 0.05$).

Cone penetrometry sampling

Soil samples were collected from 24 locations along eight transects at LAAP using CPT (Figure 5). Depths of penetration were through both Upper and Lower terraces. Generally, the penetrations were about 15 m (50 ft) deep and reached total depth in the Cane River Formation (the confining layer). Locations were determined to meet the following sampling objectives: (a) to ensure sampling from highest concentrations to zero concentration in all four cardinal directions from the source (original lagoons), (b) to provide samples for biomarker development along the leading edge of the contaminant plume, and (c) to refine the vertical and lateral definition of the contamination. Sampling on the Area P cap was not permitted because of concern that the integrity of the cap would be compromised. Previously collected CPT data were used to stratify the various lithologies and to locate new CPT sites (Edris, unpublished data from 1994). TNT and RDX concentrations from groundwater sampling to the date of the event were contoured to

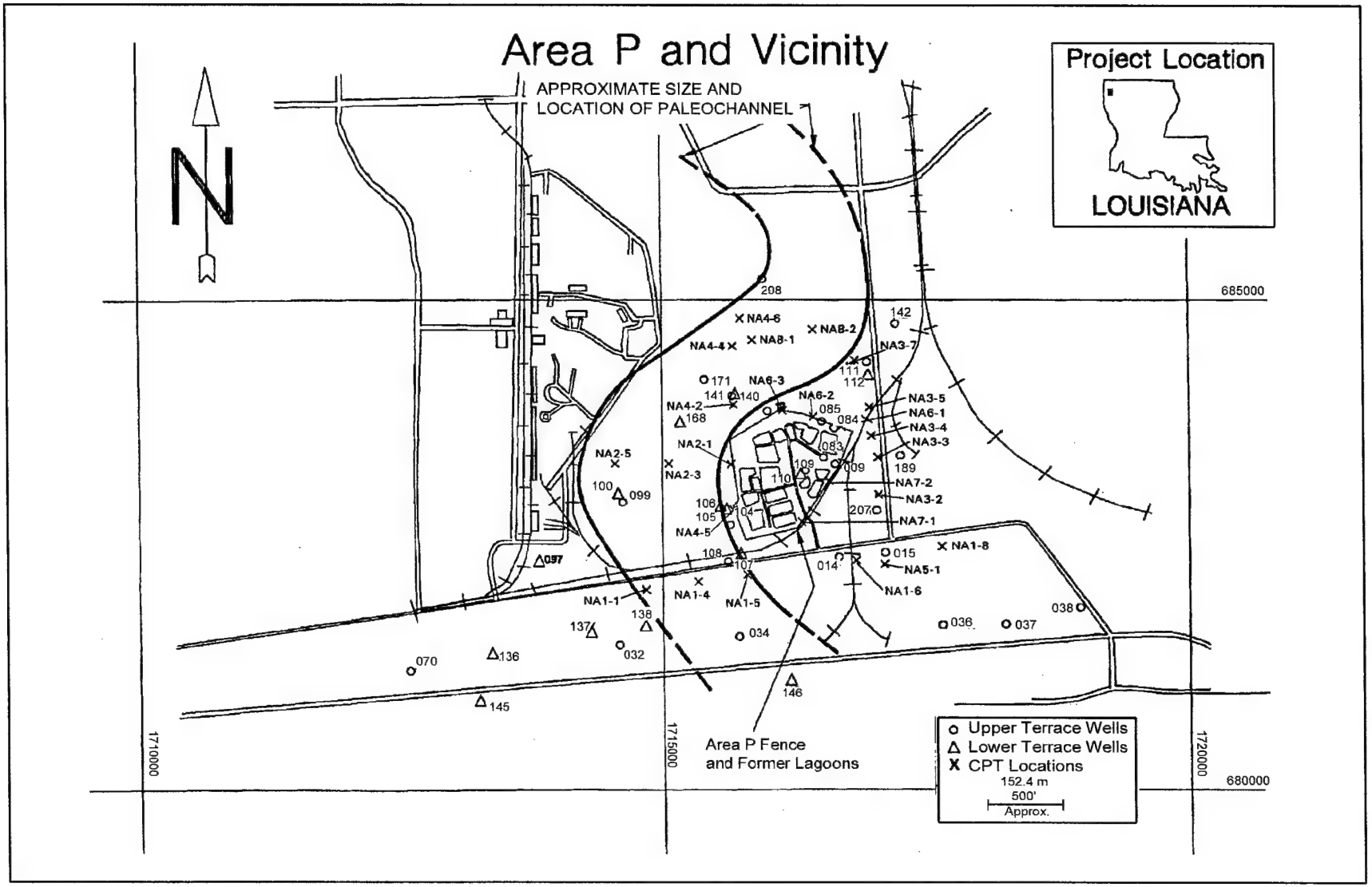

Figure 5. Locations of cone penetrometry sampling sites at LAAP

identify "hot spots" prior to establishing transects. The first punch on a given transect was used to stratify the site by measuring resistivity to penetration. The strata were defined in terms of lithology and substrate conducive to microbial processes. Subsequent punches were used to collect soil samples with a split-spoon sampler (45.7 cm, or 18 in.) that had been sterilized to minimize contamination. Typically, at least three depths, surface, middepth, and just above bedrock, were sampled. Sampling along each transect was restricted to one or two locations before proceeding to the next transect. This was done to allow time to send the samples to the laboratory and obtain explosives-concentration data upon which to base selection of the next location along that transect. Vertical profiles of soil samples were collected at five locations. Depending upon the depth of the CPT hole, from 6 to 14 samples were collected for each vertical profile. Up to 400 g of soils were collected in the sampler.

A thin vertical slice of the soil in the split-spoon was removed by opening the sampler slightly and inserting a sterile spatula into one end of the core to a depth of approximately 1 cm and running the spatula along the length of the core. This subsample was mixed and analyzed for TNT and RDX using a field-test kit (Ensys, Inc., Research Triangle Park, NC) (Jenkins 1990; Walsh and Jenkins 1991). The remainder of the core sample was removed by fully opening the sampler and sliding the contents into 1-L Ziploc bags (DowBrands L.P., Indianapolis, IN). The bags were sealed immediately, labeled as to exact location and depth, and stored on ice. Between uses, the sampler was scrubbed and sterilized for 10 min in a solution containing 20-percent household bleach and 0.01-percent detergent, followed by rinsing with a steam sprayer. Explosives and geochemical parameters, including permeability, were measured on these samples that were also used for biomarker research. Hydraulic conductivity was based on results of standard sieve analysis.

Soil samples were analyzed for explosives and transformation products of explosives by Method 8330 (EPA 1994). The difference between the procedure for soils and for groundwater was the requirement for extracting the soil prior to injection into the HPLC. Soils are extracted with acetonitrile using sonication. Analytical standards and analytes were the same as for analysis of groundwater samples. Soil samples were also analyzed for pH (Mehlich 1984), nitrate nitrogen, nitrite nitrogen, total Kjeldahl nitrogen, phosphorus, sulfate, and total organic carbon (TOC) (American Public Health Association 1985). Hydraulic conductivity was measured by standard sieve methods, and soils were classified according to the U.S. Army Corps of Engineers Unified Soil Classification System (1960). Particle-size distribution in the <2-mm fraction was determined by the methods of Day (1956) as modified by Patrick (1958).

Surface soil sampling

Surface soil samples were collected from three locations around Area P (Figure 6). Samples were collected using a clean shovel, stored on ice in sterile containers, and analyzed as described for CPT soil samples. Data from these

samples were integrated into the CPT data. Three surface soil samples were also taken from the Area P cap. These samples were analyzed for hydraulic conductivity as described for CPT samples.

Materials and Methods: JAAP

Groundwater monitoring

The initial monitoring plan was designed to sample 11 wells constructed at Site L1 during the RIs (Dames and Moore, Inc. 1993). The monitoring plan called for monthly sampling for 9 months. The wells were sounded to determine depth and to ensure that no obstructions were present. The measurements were also used to determine the length of dedicated tubing needed to sample each well. The existing analytical data were reviewed to establish a well-sampling order from least to greatest contaminant concentration. Sample collection and analysis followed the procedures established at the LAAP except that small well volumes necessitated the use of a technique employed for MW109U at LAAP whereby the wells were evacuated and allowed to recharge three consecutive times and then sampled.

Because of seasonally low-water levels in the summer of 1997, three wells (MW171, MW175, and MW176) were dry. These wells were consequently dropped from the monitoring program. Three new wells (WES1, WES2, and WES3) were completed (July 1997) in the bedrock and integrated into the sampling plan. These wells were located to contribute to the vertical and northern definition of the contamination. The new wells were drilled to a depth of 20 ft, which placed them into the Silurian age, dolomitic sandstone. The wells were 4 in. in diameter and completed with 20 ft of 10-slot polyvinyl chloride (PVC) screen. A standard sand filter pack was used to prevent the migration of fine silts and sands into the well bore. After development by airlifting techniques, each well was tested for yield. Yields were approximately 5 gal per minute for WES1 and WES2 and approximately 8 gal per minute for WES3.

Cone penetrometry sampling

At JAAP, seven transects radiating from MW131 were sampled by CPT (Figure 4). The MW131 was selected as the center because it had exhibited the highest concentrations of TNT and RDX (Table 1). The first location on Transect 1 was sampled; the samples were shipped to the laboratory; and the first location of Transect 2 was sampled. By the time one location on each transect had been sampled, analytical results for the first sample on the first transect were available. If results indicated contamination, the next location on that transect was positioned farther from MW131 in an attempt to locate the lateral extent of the contamination. If results indicated no contamination, no additional samples were taken on that transect, or the next point was moved in toward MW131. Three sites were sampled at multiple-depth intervals to obtain a vertical profile of

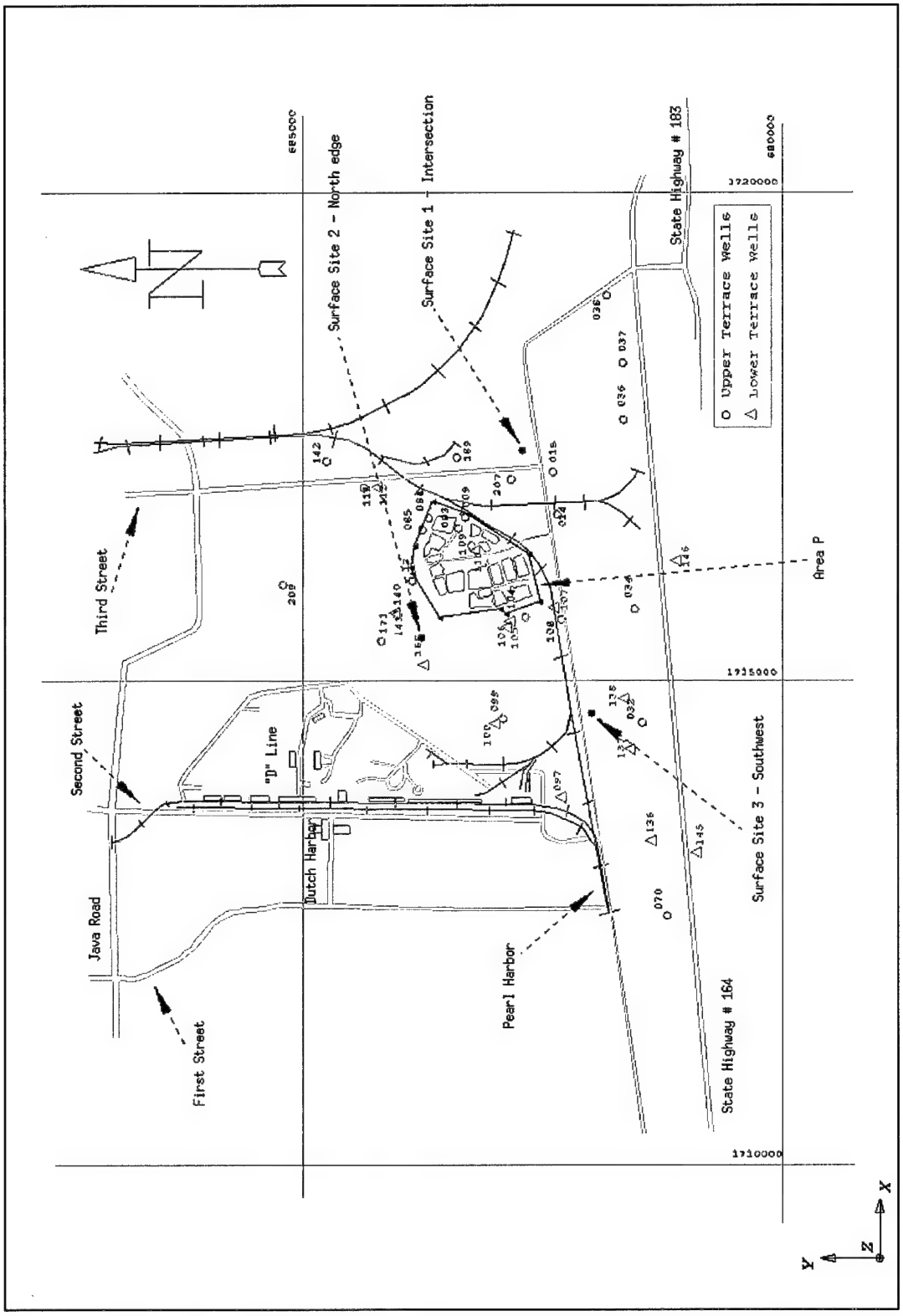

Figure 6. Locations of surface soil samples collected at LAAP

discrete samples. As at LAAP, each penetration was to bedrock, and the first punch on a given transect was used to stratify the site. Typically, at least three depths were sampled: surface, middepth, and just above bedrock. Soils were analyzed as described for LAAP.

Surface soil sampling

To further characterize the extent of explosives contamination at JAAP, a series of surface cores were taken in two perpendicular transects crossing the ridge and furrow system (Figure 7). Individual surface cores, designated "JS" followed by the sample number, were taken at approximately 40-ft intervals. The exact length of the sample intervals was varied to obtain materials from both ridges and furrows. Surface soil samples were collected as follows:

a. The sample coring devices were 2-in.- (5.08-cm-) diam by 28-in.- (72.2-cm-) length sections of PVC pipe. Individual coring devices were soaked in bleach, washed with a steam cleaner, and checked for sterility, as for the stainless steel split-spoon used for collecting the vertical profile samples.

b. A threaded adapter was attached to the CPT truck to hold the PVC coring device in place. The CPT pushed the coring device into the soil to the depth of the device (28 in.) and then retrieved the device from the soil. The top and bottom of the sample were marked on the coring device, and the ends of the device were capped. The coring devices with samples were stored on ice until analyzed.

c. Coring devices were scored along the length of two sides using a table saw, split apart under aseptic conditions, and the soil removed and homogenized as described for CPT vertical profile samples.

d. Surface samples were analyzed according to procedures described for CPT samples. Each sample was allocated as follows:

Task	Wet Weight, g
Explosives analyses	10
Particle size	70
DNA and lipid biomarkers	60
Phytoremediation	1
Geochemistry	132
Other analyses	60
Total	**333**

Several additional samples designated "PC" were taken from banks of Prairie Creek using the same procedures except for manual pushing (Figure 7).

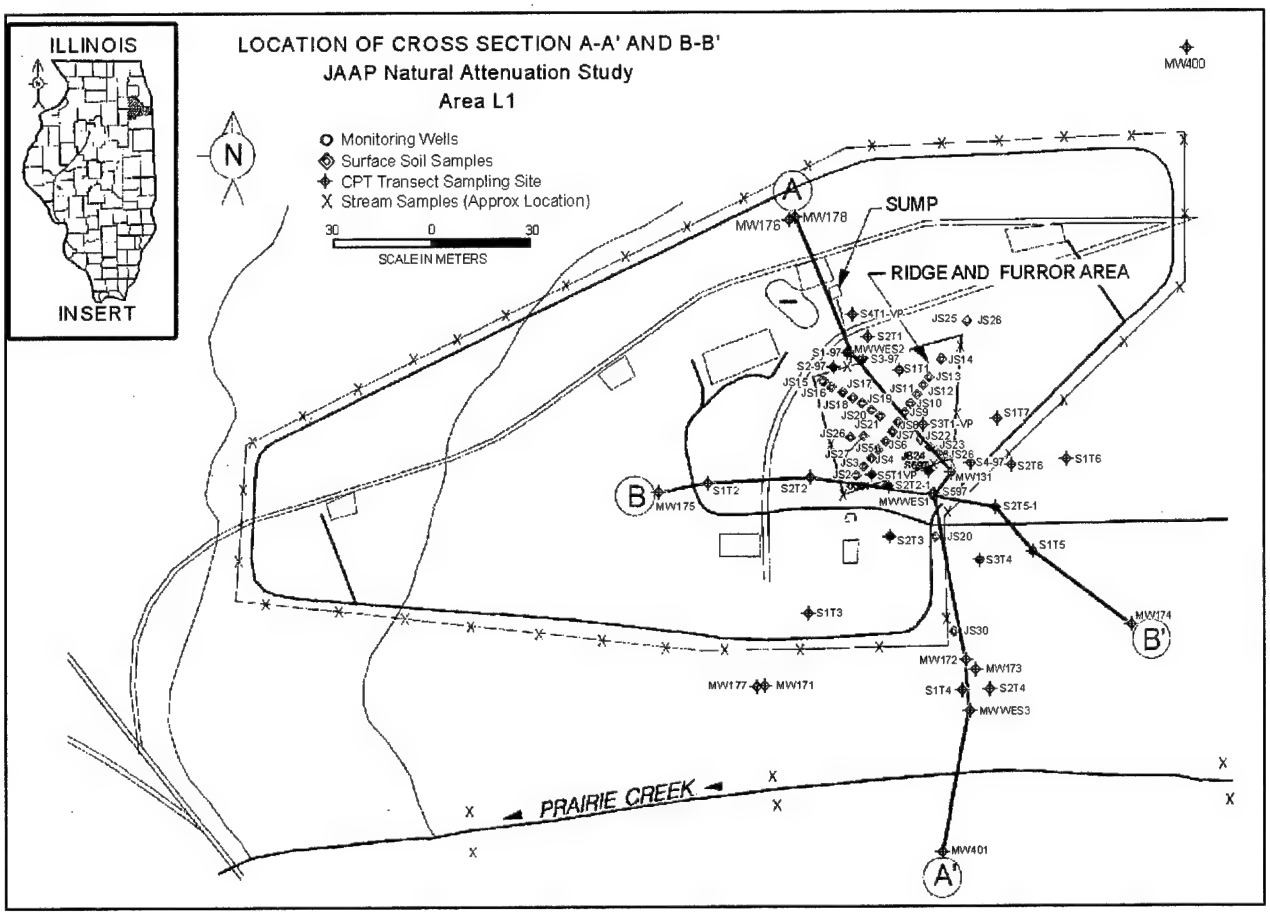

Figure 7. Geological transects, surface soil-sampling location (JS), and Prairie Creek soil-sampling locations (PC) at JAAP

Statistical analyses

Available historical data were combined with 1997-1998 contaminant concentration data (omitting Round 9, January 1998) for trend analysis. Trend analysis was not attempted unless the data for a given well included at least two detectable concentrations. Trend analysis was performed using the SAS LIFEREG procedure (SAS Institute, Inc. 1988) assuming either a normal or a lognormal distribution. The LIFEREG procedure fits a regression line by MLE and is particularly well suited to analysis of censored data such as those with below detection limit observations. LIFEREG incorporates probabilities below detection limit for the assumed distribution and does not require prior reconstitution of the censored data (e.g., using one-half the detection limit). However, a simulation study in progress has shown the LIFEREG procedure to have a high Type I error rate[1] in some circumstances. Therefore, trend analysis was also performed by OLS regression using linear, logarithmic, and quadratic models in the SAS REG procedure, following substitution of one-half detection limit for less than detection limit observations. Trends were not considered valid unless

[1] A Type I error is made by concluding an effect when none actually exists.

significant ($P < 0.05$) by at least one of the MLE models and one of the OLS models.

Results of Groundwater Monitoring: LAAP

Historical data

Observed trends over time. The results of the statistical trend analysis are displayed in Table 2 (Upper Terrace) and Table 3 (Lower Terrace). The 2,6-DNT, RDX, and tetryl showed no discernible trends over time in either terrace. Several explosives had statistically significant increasing trends in concentration over time in the more highly contaminated Inner Zone of the Lower Terrace. These included 1,3,5-trinitrobenzene (TNB), 1,3-dinitrobenzene (DNB), TNT, 2,4DNT, and 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX); 2,4DNT was also increasing over time in the Inner Zone of the Upper Terrace. In contrast, HMX and NB were statistically decreasing over time in the Inner Zone of the Upper Terrace, whereas no explosives showed decreasing trends over time in the Inner Zone of the Lower Terrace. In the less contaminated Outer Zones, all explosives had stable or decreasing concentrations over time. TNB and DNB were statistically decreasing over time in the Outer Zone of the Upper Terrace; TNB, TNT, and HMX were statistically decreasing over time in the Outer Zone of the Lower Terrace.

Data variability. The extreme variability of the historic contaminant concentration data for wells in Area P makes it difficult to discern trends in concentration over time. Observed concentrations of some explosives span several orders of magnitude in the Inner Zone of both terraces. Data variability, expressed as coefficient of variation (CV), is shown in Table 4.

General trends. A general summary of trend analysis of historical data for Area P wells is as follows:

a. Patchy contaminant concentration data, 1986-1995, for nine explosives from 66 wells.

b. Data used to map a highly contaminated Inner Zone and a less contaminated Outer Zone for each explosive in each terrace.

c. Statistical trend analysis included the following:

Normality testing (untransformed and log-transformed data).
Regression analysis (linear and quadratic).
Lack-of-fit analysis of regression models.
Nonparametric rank correlation if data were non-normal.

Observed trends may be summarized as follows:

Table 2
Summary of Trends in Explosives Concentrations over Time in Area P, Upper Terrace

	Inner Zone			Outer Zone		
Explosive	**N**	**Statistical Model**	**Trend**	**N**	**Statistical Model**	**Trend**
TNB	26	Log-linear F = 1.04, P = 0.3184	None	36	Log-quadratic F = 4.64, P = 0.0168	Generally decreasing
DNB	23	Log-linear F = 1.67, P = 0.2101	None	25	Nonparametric ρ = -0.42, P = 0.0371	Decreasing
TNT	28	Log-linear F = 0.36, P = 0.5523	None	5	Log-linear F = 3.59, P = 0.1545	None
2,4DNT	27	Log-linear F = 4.44, P = 0.0452	Increasing	0	--	--
2,6DNT	8	Log-linear F = 3.45, P = 0.1128	None	11	Nonparametric ρ = -0.30, P = 0.3728	None
HMX	23	Linear F = 4.37, P = 0.0488	Decreasing	9	Log-linear F = 2.04, P = 0.1960	None
NB	8	Log-quadratic F = 7.00, P = 0.0356	Decreasing after 1990	0	--	--
RDX	36	Log-linear F = 0.13, P = 0.7240	None	8	Log-linear F = 0.03, P = 0.8773	None
Tetryl	10	Nonparametric ρ = 0.05, P = 0.8868	None	10	Log-linear F = 0.08, P = 0.7909	None

Table 3
Summary of Trends in Explosives Concentrations over Time in Area P, Lower Terrace

	Inner Zone			Outer Zone		
Explosive	**N**	**Statistical Model**	**Trend**	**N**	**Statistical Model**	**Trend**
TNB	20	Log-linear F = 13.88, P = 0.0015	Increasing	35	Log-linear F = 6.17, P = 0.0182	Decreasing
DNB	22	Log-linear F = 9.83, P = 0.0052	Increasing	19	Nonparametric ρ = -0.13, P = 0.6086	None[1]
TNT	23	Log-linear F = 6.36, P = 0.0198	Increasing	17	Nonparametric ρ = -0.58, P = 0.0148	Decreasing[1]
2,4DNT	21	Log-linear F = 4.84, P = 0.0403	Increasing	22	Nonparametric ρ = 0.28, P = 0.2104	None[1]
2,6DNT	5	Log-linear F = 0.47, P = 0.5434	None[1]	18	Quadratic F = 2.97, P = 0.0821	None[1]
HMX	22	Log-quadratic F = 3.55, P = 0.0489	Increasing after 1989	28	Log-quadratic F = 3.70, P = 0.0392	Decreasing after 1989
NB	13	Nonparametric ρ = 0.13, P = 0.6807	None[1]	14	Nonparametric ρ = -0.47, P = 0.0926	None[1]
RDX	44	Log-linear F = 2.81, P = 0.1008	None	17	Log-linear F = 3.18, P = 0.0949	None
Tetryl	3	Nonparametric ρ = -0.50, P = 0.6667	None[1]	7	Nonparametric ρ = -0.15, P = 0.7530	None

[1] No data after 1990.

Table 4
Range and Coefficient of Variation (CV)[1] of Concentration Data from Area P Wells, 1986-1995

Explosive	Upper Terrace			Lower Terrace		
	Inner Zone		Outer Zone[2]	Inner Zone		Outer Zone[1]
	Range (Orders of Magnitude)	CV	CV	Range (Orders of Magnitude)	CV	CV
TNB	3	1.66	1.15	3	1.63	1.23
DNB	3	2.08	0.48	2	1.21	0.62
TNT	4	1.31	0.39	3	1.96	1.27
2,4DNT	2	1.39	--	2	1.11	1.12
2,6DNT	2	2.04	0.71	1	0.64	0.77
HMX	3	1.10	0.59	2	1.72	0.80
NB	3	1.59	--	3	2.16	0.84
RDX	4	1.32	1.26	3	1.68	0.88
Tetryl	2	1.44	1.24	1	1.62	1.01

[1] The coefficient of variation is the ratio of the standard deviation to the mean.
[2] The range of data for the Outer Zone was always <1 order of magnitude.

a. Extreme data variability (up to 4 orders of magnitude in a sample year) made trends difficult to identify.

b. No significant trends over time for RDX, tetryl, and 2,6-DNT.

c. TNT, TNB, DNB, 2,4-DNT, and HMX increasing over time in the Inner Zone of the Lower Terrace aquifer.

d. TNT, TNB, and HMX decreasing over time in the Outer Zone of the Lower Terrace aquifer.

e. Fewer trends in the Upper Terrace aquifer.

f. All explosives below detection limit in all water supply wells.

Data quality

Stabilization of explosives concentrations. TNT concentration was inversely correlated with DO concentrations over pumping time in MW100L and MW168L (Figure 8, Table 5). In MW110L, the relationship was inverse (r^2 = -0.49) but not significant (P = 0.22). RDX concentration was inversely correlated with DO in MW168L, but negative correlations in the other two wells were not significant (MW100L, r^2 = -0.614, P = 0.078; MW110L, r^2 = -0.414,

P = 0.307). Several transformation products of TNT also exhibited significant correlations with DO concentrations over time (Table 5). These were inverse except in MW110L where the monoamino transformation products of TNT, 4ADNT and 2ADNT, and DNA decreased as DO decreased. Perhaps these products are more stable under the oxidized conditions at the well head. The DO typically stabilized when approximately three well volumes had been discharged (Table 6). Results of a paired t-test of data from 10 wells indicated no significant difference between three well volumes and the actual volume pumped using the micropurge technique. Therefore, for LAAP, bailing of three well volumes is roughly equivalent to stabilization of DO for determining when to collect samples.

Sample preservation. No differences in preservatives were observed for any analyte even after accounting for concentration differences between wells. The results did not change when ranks rather than raw data were used in the analysis. However, when the data for each analyte were ranked within each well and used in a one-way analysis of variance (ANOVA), a significant difference was observed for TNT ($F = 4.10$, $P = 0.0340$, $N = 21$, $NaH_2SO_4 > HgCl_2$ with no preservative intermediate). Therefore, NaH_2SO_4 was used as a preservative for all subsequent samples.

Precision and accuracy. Results of paired t-tests indicated no differences between analyte concentrations for duplicate samples. Therefore, the precision of analytical methods was good. The accuracy of the analytical methods as demonstrated by recoveries of explosives spiked into contaminated groundwater and into

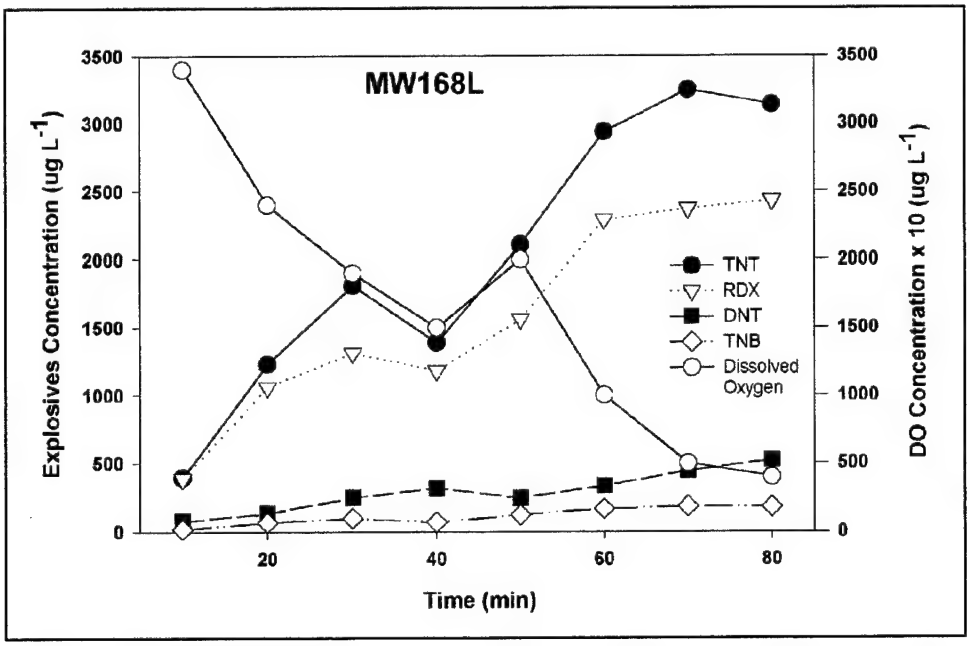

Figure 8. Relationship between dissolved oxygen and explosives concentration over pumping time in MW168L, LAAP

Table 5
Pearson Product Moment Correlations for Dissolved Oxygen and Explosives Concentrations over Pumping Time (P < 0.05) at LAAP

Monitoring Wells	R-square[1]	P[2]	n[3]
MW100L			
TNT	-0.755	0.019	9
2,4DNT	-0.870	0.0023	9
MW110L			
4ADNT	0.864	0.0057	8
2ADNT	0.839	0.0092	8
DNA	0.910	0.0017	8
MW168L			
TNT	-0.937	0.00061	8
RDX	-0.948	0.00033	8
2ADNT	-0.970	0.000067	8
TNB	-0.920	0.0012	8

[1] Correlation coefficient.
[2] Probability value.
[3] Number of samples.

Table 6
Comparison of Three Well Volumes with Actual Volume of Water Pumped Using Micropurge Technique[1]

Well Number	Three Well Volumes, L	Actual Volume Pumped, L
MW104U	132.97	227.30
MW100L	407.77	409.14
MW110L	496.42	500.06
MW168L	381.18	340.95
MW012L	22.71	250.03
MW083U	25.23	113.65
MW111U	230.48	250.03
MW189U	35.46	68.19
MW097L	354.59	431.87
MW108L	505.28	727.35

[1] Based upon analysis of 10 contaminated wells at LAAP.

reversed osmosis (RO) water blanks was good. Pooled means were typically within two standard error units of 100 percent (Table 7). No analytes were detected in unspiked blanks.

Table 7
Accuracy of Explosives Analyses at LAAP Based upon Percent Recoveries of Spikes into Contaminated Groundwater Samples (number of samples spiked = 30) and into Reverse Osmosis Water (number of samples = 24)

	Contaminated Groundwater			RO Water Blanks		
Analyte	Pooled Mean % Recovery	Pooled Standard Error	Range	Pooled Mean % Recovery	Pooled Standard Error	Range
TNT	101.0	1.68	93-118	102.4	1.05	94-114
RDX	101.4	2.10	89-119	106.8	1.29	97-119
HMX	101.8	1.14	97-114	103.2	1.17	93-113
TNB	100.5	1.22	89-113	103.3	1.08	94-112
4ADNT	99.4	1.60	88-116	102.6	1.15	94-113
2,4DNT	98.5	1.91	92-116	100.8	0.820	96-110

Split-sample analyses. Results of a paired t-test indicated no difference between analytical results achieved by the two independent laboratories for six of the seven analytes. CRREL values for HMX tended to be slightly greater than WES values ($P = 0.002$).

General groundwater quality

Geochemistry. Concentrations of calcium and magnesium suggest that LAAP groundwater is generally soft, i.e., sum of calcium and magnesium concentrations were less than 50 mg L^{-1} (Chapter 2 Appendix Table 1). A few wells (MW034U, MW099U, and MW138L) exhibited hard water (sum of calcium and magnesium concentrations greater than 200 mg L^{-1}). Four wells (MW012U, MW038U, MW110L, and MW112L) exhibited total iron concentrations above the drinking water standard of 0.3 mg L^{-1}. Concentrations of manganese, which behaves geochemically in a manner similar to iron, in about half of the wells exceeded the drinking water regulatory limit (0.05 mg L^{-1}) with highest concentrations around 0.5 mg L^{-1}. About half of these were deep wells. Values of 2 to 3 mg L^{-1} are common in deep water wells. Nitrate/nitrite nitrogen values were generally low. The range for natural waters is 0.1 to 10 mg L^{-1} (Driscoll 1986). Most values were much less than 10 mg L^{-1}. Four wells exhibited values greater than 10 mg L^{-1} (MW100L, MW109U, MW141L, MW168L), but the values were well below the level at which water becomes unsuitable for domestic water supply (45 mg L^{-1}, Driscoll 1986). No values exceeded 20 mg L^{-1}, which is considered harmful to infants. Sulfate concentrations were relatively low. Most values were less than 10 mg L^{-1}. Chloride concentrations less than 150 mg L^{-1} are considered

satisfactory for most purposes. Only a few values exceeded 150 mg L^{-1}. Reduced iron was sampled in only 12 wells in only two sampling rounds. About half of these wells exhibited reduced iron; most values were <1 mg L^{-1}. No methane was observed in these same samples.

Explosives and their transformation products

TNT, RDX, HMX, and tetryl. Concentrations of TNT ranged from non-detectable (<0.20 μg L^{-1}) in wells peripheral to Area P to a high of 15,000 μg L^{-1} in MW104U (Chapter 2 Appendix Table 2). Highest concentrations of RDX and HMX, 25,000[1] and 1,900 μg L^{-1}, respectively, were observed in the same well. This well is located on the east-southeastern border of Area P (Figure 2). No tetryl was detected in any wells. The wells in which the most analytes were consistently detected were the five upper terrace wells, MW083U, MW085U, MW104U, MW109U, and MW140U, and six lower terrace wells, MW100L, MW105L, MW110L, MW112L, MW141L, and MW168L. Concentrations were generally higher in the upper than in the lower terrace. Variability in the concentration data was generally lower in the upper than in the lower terrace.

Transformation products. The highest concentration of a TNT transformation product was exhibited by TNB. TNB, DNB, and NB are photodegradation products of TNT and may have resulted from the prolonged exposure of the wastewater in the former lagoons to sunlight. TNB was detected in most wells in which TNT was detected and ranged in concentration up to 10,600 μg L^{-1} (MW085U). Concentrations of DNB were detected as frequently as TNB, but the highest concentration was less (559 μg L^{-1} in MW104U). The NB was detected sporadically at very low levels (maximum of 1.56 μg L^{-1} in MW107L). The dinitrotoluene, 2,4DNT, was frequently detected at relatively high levels (up to 560 μg L^{-1} in MW141L). This compound is often a part of the explosive loaded into shells and casings; therefore, 2,4DNT may have been introduced with the initial lagoon wastewater. The 2,6DNT was detected rarely and at very low concentrations. The monoamino transformation products of TNT, 2ADNT and 4ADNT, were widely detected. The number of consistent detections was greater for 2ADNT (13 wells as opposed to 7 wells for 4ADNT), but concentrations were higher for 4ADNT (up to 357 μg L^{-1}) compared with 2ADNT (up to 217 μg L^{-1}). The presence of these compounds is an indication of abiotic or microbial transformation processes in the site. The monoamino transformation products are susceptible to immobilization processes. The diamino transformation product, 2,4DANT, was detected in two wells (MW109U and MW110L) at a high of 77.2 μg L^{-1} and sporadically in several other wells. The 3,5DNA (detected in 11 wells at concentrations up to 306 μg L^{-1}) is a photodegradation product of TNB. No AZOXY dimers were detected. The RDX transformation products, MNX and TNX, were rarely detected, and DNX was not detected. The HMX transformation product, MN-HMX, was rarely detected.

[1] Aqueous solubilities of RDX and HMX at 20 °C are approximately 42,000 and 2,600 μg L^{-1}, respectively (Sikka et al. 1980 and Spanggord et al. 1982, respectively).

Picric acid. As indicated below, six wells exhibited picric acid concentrations above detection limits (5 μg L^{-1}). One of the groundwater samples collected by CPT also contained picric acid. The sample, NA2-1-33, contained 552 μg L^{1}.

Monitoring Well	Concentration, μg L^{-1}
MW168L	2,320.0
MW141L	1,980.0
MW085U	507.0
MW112L	62.6
MW140U	58.2
MW171U	14.0

Trends in explosives concentrations. Over the 2 years, significant declines in concentrations occurred in 9 of the 11 wells in which analytes were consistently detected (Table 8). Most of the analytes decreased in concentration in MW083U, MW085U, MW140U, and MW105L (Figure 9). Except for MW141L and MW168L, all 11 wells exhibited significant decreases in concentrations of at least one analyte. Increasing concentrations of three contaminants, including RDX, were observed in MW100L. Increases in RDX were also observed in MW141L and MW168L. Overall, significant declines occurred in 44 percent of all cases analyzed, while significant increases occurred in 7.5 percent of the cases (Table 9). No significant trends were observed in 48 percent of the cases. Results of Spearman's rank correlations confirmed 80 percent of the significant regression trends observed in the 11 wells. Collective results in these data provide evidence for natural attenuation of explosives and their derivatives in many of the most contaminated Area P wells.

Correlations between geochemical parameters and explosives concentrations. Few significant correlations were noted for Ca, Cl, Fe, NO_2/NO_3, and SO_4 (Table 10). More significant contaminant correlations occurred with Mg and Mn, and these were predominantly positive. The TOC was significantly correlated with the most contaminants in the most wells, and all of these correlations were positive. Almost all significant correlations with water level, on the other hand, were negative. Most significant correlations (both positive and negative) occurred in MW085U, MW109U, MW140U, MW105L, and MW083U. Contaminant concentrations in MW168L were not significantly correlated with any water parameter. These results suggest that none of these geochemical characteristics exert a significant effect upon explosives concentrations. Therefore, monitoring these parameters did not provide evidence for natural attenuation of the explosives at LAAP.

Table 8
Regression Statistics for Significant (P < 0.05, N = 12) Contaminant Concentration Trends over 2 Years in 11 Wells at LAAP

Explosive	Well	MLE[1] Regression Statistics[2]		OLS Regression Statistics[3]				Trend (Slope)
		X^2	P	Model	F	P	R-square	
TNT	083U	31.891	0.0001	Linear	26.576	0.0004	0.727	-
	085U	35.189	0.0001	Linear	29.324	0.0003	0.746	-
	100L	70.053	0.0001	Linear	58.378	0.0001	0.854	+
	105L	5.774	0.0163	Quadratic	6.098	0.0212	0.575	+ then -
	109U	31.699	0.0001	Linear	26.417	0.0004	0.725	-
	140U	36.085	0.0001	Linear	30.071	0.0003	0.750	-
RDX	083U	136.677	0.0001	Linear	113.898	0.0001	0.919	-
	085U	23.146	0.0001	Linear	19.289	0.0014	0.659	-
	100L	56.415	0.0001	Linear	47.012	0.0001	0.825	+
	105L	6.270	0.0123	Linear	5.225	0.0453	0.343	-
	140U	53.554	0.0001	Linear	44.628	0.0001	0.817	-
	141L	31.900	0.0001	Linear	26.583	0.0004	0.727	+
	168L	21.337	0.0001	Linear	17.781	0.0018	0.640	+
HMX	083U	34.198	0.0001	Linear	28.499	0.0003	0.740	-
	085U	13.865	0.0002	Linear	11.554	0.0068	0.536	-
	105L	17.902	0.0001	Linear	14.918	0.0031	0.599	-
	112L	4.029	0.0447	Quadratic	9.334	0.0064	0.675	- then +
	140U	16.725	0.0001	Linear	13.938	0.0039	0.582	-
TNB	083U	10.168	0.0014	Linear	8.474	0.0155	0.459	-
	085U	8.166	0.0043	Linear	6.805	0.0261	0.405	-
	100L	9.546	0.0020	Linear	7.955	0.0181	0.443	-
	105L	20.323	0.0001	Linear	16.936	0.0021	0.629	-
	109U	24.740	0.0001	Linear	20.617	0.0011	0.673	-
	112L	13.149	0.0003	Linear	10.957	0.0079	0.522	-

(Continued)

[1] Maximum likelihood estimation according to SAS LIFEREG (SAS Institute, Inc. 1988).
[2] Normal distribution model.
[3] Ordinary least squares according to SAS REG (SAS Institute, Inc. 1988).

Table 8 (Concluded)

Explosive	Well	MLE[1] Regression Statistics[2]		OLS Regression Statistics[3]				Trend (Slope)
		X^2	P	Model	F	P	R-square	
TNB	140U	11.539	0.0007	Linear	9.616	0.0112	0.490	-
2ADNT	083U	11.377	0.0007	Linear	9.481	0.0117	0.487	-
	085U	6.901	0.0086	Linear	6.216	0.0318	0.383	-
	105L	24.746	0.0001	Linear	22.454	0.0008	0.692	-
	110L	5.769	0.0163	Log_{10}	5.094	0.0476	0.338	-
	112L	15.633	0.0001	Linear	13.028	0.0048	0.566	-
	140U	9.583	0.0020	Linear	7.986	0.0180	0.444	-
4ADNT	083U	16.562	0.0001	Linear	13.802	0.0040	0.580	-
	105L	4.394	0.0361	Linear	9.585	0.0113	0.489	-
	110L	9.928	0.0016	Linear	8.274	0.0165	0.453	-
2,4DNT	085U	24.037	0.0001	Linear	20.031	0.0012	0.667	-
	100L	9.592	0.0020	Linear	7.993	0.0179	0.444	+
	109U	26.480	0.0001	Linear	22.067	0.0008	0.688	-
	140U	8.483	0.0036	Linear	7.069	0.0239	0.414	-
3,5DNA	083U	31.889	0.0001	Linear	26.574	0.0004	0.727	-
	085U	15.852	0.0001	Linear	13.210	0.0046	0.569	-
	109U	30.222	0.0001	Linear	25.185	0.0005	0.716	-
DNB	083U	19.516	0.0001	Linear	16.263	0.0024	0.619	-
	085U	49.116	0.0001	Linear	40.930	0.0001	0.804	-
	100L	7.223	0.0072	Linear	6.019	0.0341	0.376	-
	104U	9.054	0.0026	Linear	7.545	0.0206	0.430	-
	109U	6.424	0.0113	Linear	5.353	0.0432	0.349	+
	110L	7.180	0.0074	Linear	5.983	0.0345	0.374	-
	140U	32.747	0.0001	Linear	27.289	0.0004	0.732	-

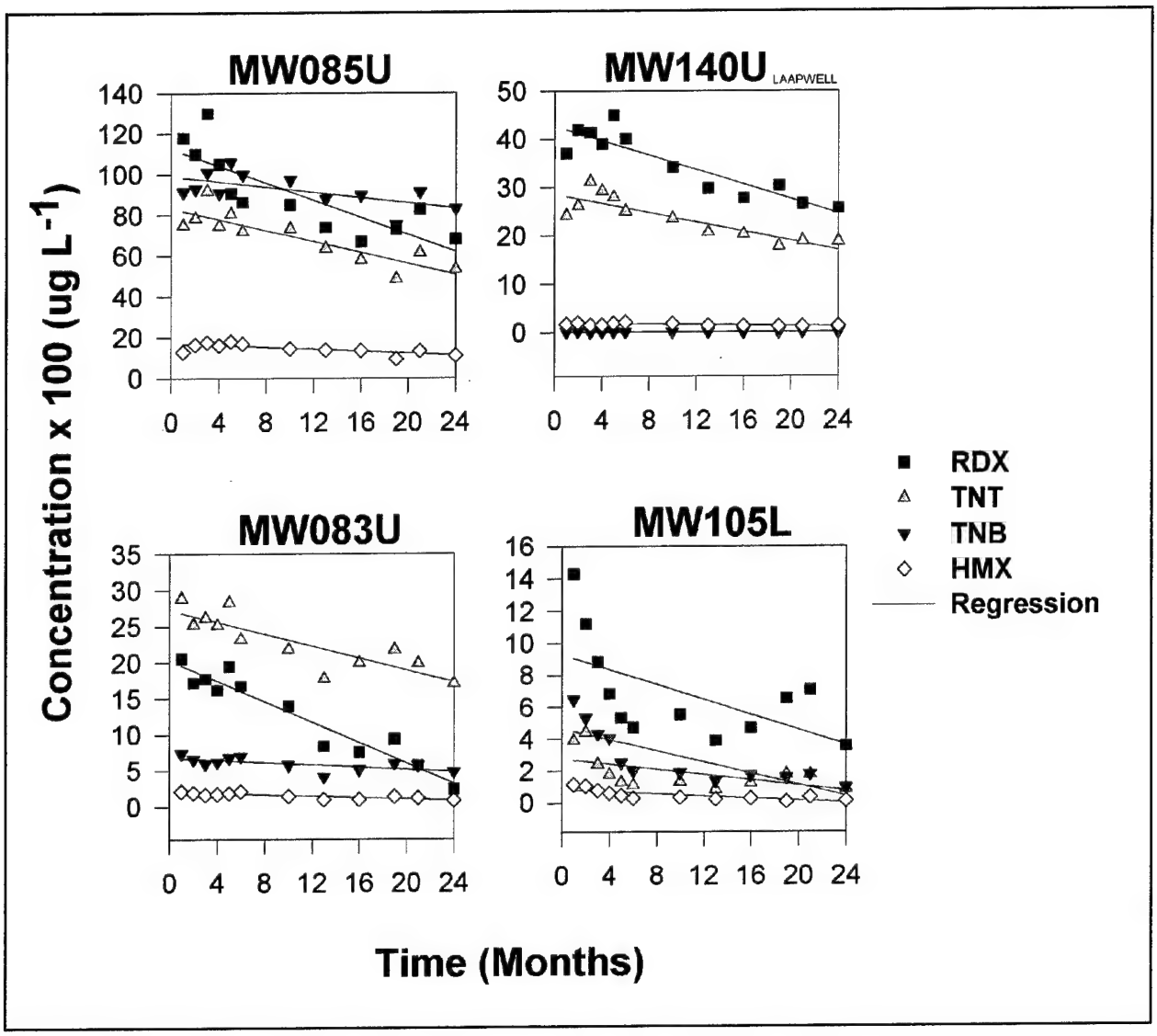

Figure 9. Trends in explosives concentration over sampling events at LAAP

Results of Cone Penetrometry: LAAP

The LAAP soils were predominantly sand to loamy sand with extremely low organic carbon content (generally <0.1 percent) (Table 11). The pH ranged from 5 to 8 with a mean of approximately 6. Nitrogen levels (total nitrogen ranged from approximately 480 to 750 mg kg^{-1}) were typical of humid timbered soils such as those found at LAAP (Millar, Turk, and Foth 1958). Hydraulic conductivities are given in Chapter 5, Numerical Modeling.

The CPT data defined the surficial deposits of Area P as predominately silts, clays, and silty sands and confirmed the presence of a paleochannel located just west of the project site (Figures 5, 10 through 13). CPT punches along Transect 2

Table 9
Summary of Significantly Increasing (+) and Decreasing (-) Trends in Contaminant Concentrations over Time for 11 Wells at LAAP

Explosive	Well										
	MW083U	MW085U	MW104U	MW109U	MW140U	MW100L	MW105L	MW110L	MW112L	MW141L	MW168L
TNT	-	-		-	-	+	-				
RDX	-	-			-	+	-			+	+
HMX	-	-			-	<dl	-		+		
TNB	-	-		-	-	-	-		-		
2ADNT	-	-			-		-	-	-		
4ADNT	-	<dl[1]	<dl			<dl	-	-		<dl	<dl
2,4DNT		-		-	-	+					
DNA	-	-		-							
DNB	-	-	-	+	-	-		-			

[1] <dl: at least 10 of 12 measured concentrations were less than detection limit.

Table 10
Significant Correlations (P < 0.05 for both Pearson's r and Spearman's Rho) Between Geochemical Parameters and Contaminant Concentrations at LAAP

Geochemical Parameter	Significant Positive Correlations (r, P, n)		Significant Negative Correlations (r, P, n)	
	Contaminant(s)	Well	Contaminant(s)	Well
Ca	--	--	3,5DNA (-0.938, 0.0056, 6)	140U
Cl	TNB (0.941, 0.0169, 5)	085U	RDX (-0.969, 0.0066, 5)	110L
			3,5DNA (-0.920, 0.0271, 5)	141L
Fe	2ADNT (0.901, 0.0001, 12) 3,5DNA (0.934, 0.0001, 12)	110L	TNT (-0.583, 0.0468, 12)	110L
			TNB (-0.763, 0.0063, 11) 2ADNT (-0.748, 0.0081, 11)	112L
			HMX (-0.683, 0.0144, 12)	140U
Mg	RDX (0.822, 0.0448, 6) 2ADNT (0.822, 0.0447, 6)	083U	3,5DNA (-0.935, 0.0062, 6)	104U
			TNT (-0.851, 0.0315, 6)	110L
	HMX (0.873, 0.0233, 6) DNB (0.823, 0.0442, 6) TNT (0.953, 0.0033, 6)	085U		
	DNB (0.944, 0.0159, 5)	105L		
	2ADNT (0.832, 0.0400, 6)	110L		
Mn	HMX (0.763, 0.0039, 12) RDX (0.847, 0.0005, 12) TNB (0.737, 0.0063, 12) DNB (0.941, 0.0001, 12) TNT (0.940, 0.0001, 12) 2,4DNT (0.901, 0.0001, 12) 3,5DNA (0.726, 0.0075, 12)	085U	RDX (-0.765, 0.0060, 11) TNT (-0.761, 0.0065, 11) 2ADNT (-0.812, 0.0024, 11)	100L
			TNB (-0.723, 0.0079, 12) 2,4DNT (-0.805, 0.0016, 12) 3,5DNA (-0.761, 0.0040,12)	109U
	TNB (0.700, 0.0164, 11)	100L		
	2ADNT (0.920, 0.0001, 12) 3,5DNA (0.887, 0.0001, 12)	110L		
	TNB (0.817, 0.0021, 11) 2ADNT (0.641, 0.0336, 11)	112L		
	RDX (0.655, 0.0207, 12) TNT (0.611, 0.0350, 12)	140U		
NO_2/NO_3	4ADNT (0.787, 0.0024, 12) 2ADNT (0.745, 0.0054, 12)	083U	--	--
SO4	TNB (0.830, 0.0016, 11) TNT (0.857, 0.0008, 11) 2,4DNT (0.743, 0.0087, 11) 3,5DNA (0.788, 0.0039, 11)	109U	--	--

(Sheet 1 of 3)

Table 10 (Continued)

Geochemical Parameter	Significant Positive Correlations (r, P, n)		Significant Negative Correlations (r, P, n)	
	Contaminant(s)	Well	Contaminant(s)	Well
TOC	HMX (0.729, 0.0071, 12) RDX (0.849, 0.0005, 12) TNB (0.593, 0.0423, 12) DNB (0.695, 0.0121, 12) TNT (0.771, 0.0033, 12) 4ADNT (0.728, 0.0073, 12) 2ADNT (0.744, 0.0055, 12) 3,5DNA (0.756, 0.0044, 12)	083U	--	--
	HMX (0.720, 0.0083, 12) TNB (0.802, 0.0017, 12) TNT (0.680, 0.0151, 12) 2,4DNT (0.639, 0.0252, 12) 3,5DNA (0.725, 0.0076, 12)	085U		
	DNB (0.827, 0.0017, 11)	100L		
	HMX (0.813, 0.0024, 11) RDX (0.817, 0.0021, 11) TNB (0.849, 0.0009, 11) DNB (0.697, 0.0172, 11) TNT (0.655, 0.0286, 11) 4ADNT (0.695, 0.0176, 11) 2ADNT (0.843, 0.0011, 11)	105L		
	TNB (0.593, 0.0422, 12) TNT (0.716, 0.0089, 12) 2,4DNT (0.605, 0.0373, 12) 3,5DNA (0.646, 0.0231, 12)	109U		
	DNB (0.640, 0.0251, 12)	110L		
	HMX (0.646, 0.0233, 12) RDX (0.822, 0.0010, 12) DNB (0.681, 0.0147, 12) TNT (0.702, 0.0110, 12) 2ADNT (0.727, 0.0074, 12) 2,4DNT (0.583, 0.0567, 12)	140U		
	2,4DNT (0.654, 0.0211, 12) 3,5DNA (0.775, 0.0031, 12)	141L		
Water Level	RDX (0.596, 0.0407, 12) TNT (0.629, 0.0286, 12)	100L	RDX (-0.792, 0.0021, 12) DNB (-0.819, 0.0011, 12) TNT (-0.688, 0.0135, 12)	085U

(Sheet 2 of 3)

Table 10 (Concluded)

Geochemical Parameter	Significant Positive Correlations (r, P, n)		Significant Negative Correlations (r, P, n)	
	Contaminant(s)	Well	Contaminant(s)	Well
Water Level (Cont.)	RDX (0.714, 0.0091, 12)	141L	4ADNT (-0.638, 0.0255) 2,4DNT (-0.843, 0.0006, 12) 3,5DNA (-0.726, 0.0075, 12)	085U
			TNB (-0.830, 0.0008, 12)	100L
			HMX (-0.813, 0.0013, 12) RDX (-0.804, 0.0016, 12) TNB (-0.840, 0.0006, 12) TNT (-0.678, 0.0154, 12) 4ADNT (-0.749, 0.0051, 12) 2ADNT (-0.661, 0.0192, 12)	105L
			TNB (-0.888, 0.0001, 12) TNT (-0.820, 0.0011, 12) 2,4DNT (-0.946, 0.0001, 12) 3,5DNA (-0.936, 0.0001, 12)	109U
			TNB (-0.797, 0.0019, 12)	112L
			HMX (-0.711, 0.0095, 12) RDX (-0.801, 0.0017, 12) DNB (-0.803, 0.0017, 12) TNT (-0.724, 0.0077, 12) 2,4DNT (-0.694, 0.0123, 12)	140U

(Sheet 3 of 3)

indicated the paleochannel consisted of clays to a depth of approximately 20 to 30 ft, underlain by a sequence of silts (ML) and silty sands (SM) down to a depth of 45 ft. The clays, silts, and silty sands are part of the Upper Terrace deposits that unconformably overlay the Cain River Formation, which occurs at a depth of 40 to 50 ft. The Paleochannel is interpreted to be of Pleistocene Age, since the channel incised 20 to 30 ft into the Upper Terrace deposits.

Explosives concentrations were primarily limited to TNT, RDX, TNB, and HMX with sporadic detections of DNB, 4ADNT, 2ADNT, 2,4DNT, and MNX (Table 12). The concentration of TNB at NA4-5VP-7.6 was the highest detection, 13,100 mg kg^{-1}. Maximum concentrations for TNT and RDX were 7,060 and 11,800 mg kg^{-1}, respectively, at NA4-2VP-7.7. The DNB was restricted to Transect NA4. The detection of 4ADNT was limited to one detection in Transect NA4. The 2ADNT was detected on Transect NA4, NA6, and NA7. The 2,4DNT was detected in only two transects, NA2 at 1L-18.9 and 2VP-7.7, and NA4 at three locations 1L-20.2, 5VP-6.1, and 5VP-20.5. The MNX was observed in NA4-5VP. No diamino transformation products of TNT were observed. Trends in concentration with depth were evident in the vertical profile samples (Figure 14). Generally, concentrations increased with depth until a maximum and then decreased indicating a single plume of contamination;

Table 11
Particle Size and Geochemical Composition of Select CPT Soil Samples at LAAP

Sample	Particle size, %			Texture[4]	pH	TOC[5], %	Geochemical Parameters, mg kg^{-1}			
	Sand[1]	Silt[2]	Clay[3]				NO_3-N[6]	Total Nitrogen	NH4-N[7]	Phosphorus
NA1-4-U-43FT	85	7	8	Loamy sand	7.9	0.08	9	749	2	625
NA2-1-U-25.6FT	89	5	6	Sand	5.2	0.07	6	482	3	546
NA3-5-U-24FT	89	5	6	Sand	5.5	0.05	6	634	3	478
NA3-5-6-41.7FT	93	5	2	Sand	6.2	0.09	5	689	3	521
NA3-7-U-36.4FT	91	3	6	Sand	6.2	0.09	7	489	3	516
NA3-7-L-55.9FT	IS[8]	IS	IS	IS	6	0.1	12	597	4	562
NA4-5-U-30.4FT	89	5	6	Sand	6.3	0.09	7	535	2	583
NA4-5-L-67.1FT	93	5	2	Sand	7.1	0.09	5	621	3	538
NA6-2VP-10FT	61	15	24	Sandy clay loam	5.2	0.06	5	618	5	564
NA6-2VP-20FT	79	11	10	Sandy loam	5.5	0.07	4	749	5	562
NA6-2-U-27FT	81	10	9	Loamy sand	5.4	0.1	5	599	4	428
NA6-2-34FT	87	2	11	Loamy sand	6.2	0.05	5	618	4	514
NA6-2-40.2FT	89	3	8	Sand	6.4	0.14	8	615	4	482
NA6-2-L-57.4FT	85	7	7	Loamy sand	6.1	0.08	5	563	2	486

[1] Sand is any particle >0.05 mm and <2mm.
[2] Silt falls in the range of 0.05-0.002 mm.
[3] Clay is particle <0.002 mm.
[4] According to U.S. Department of Agriculture method for naming soils based upon mechanical analyses (Buckman and Brady 1969).
[5] Total organic carbon.
[6] Nitrate nitrogen.
[7] Ammonia nitrogen.
[8] Insufficient sample.

however, at one location, NA4, a second maximum was observed indicating "fingers" of the plume, or passage through an uncontaminated layer, e.g., an impenetrable clay lens. No significant relationship (as determined by Pearson Product Moment Correlation) could be established between concentrations of TNT, RDX, and TNB and cone penetrometry tip resistance (an indicator of the kind of material penetrated) (Figure 14). However, a generally inverse relationship is evident in Figure 14.

Results using field test kits and laboratory determinations of RDX and CPT soils were in good agreement (no significant difference, P = 0.05). However, field results were generally greater than lab results for TNT, but were within an order of magnitude of laboratory results.

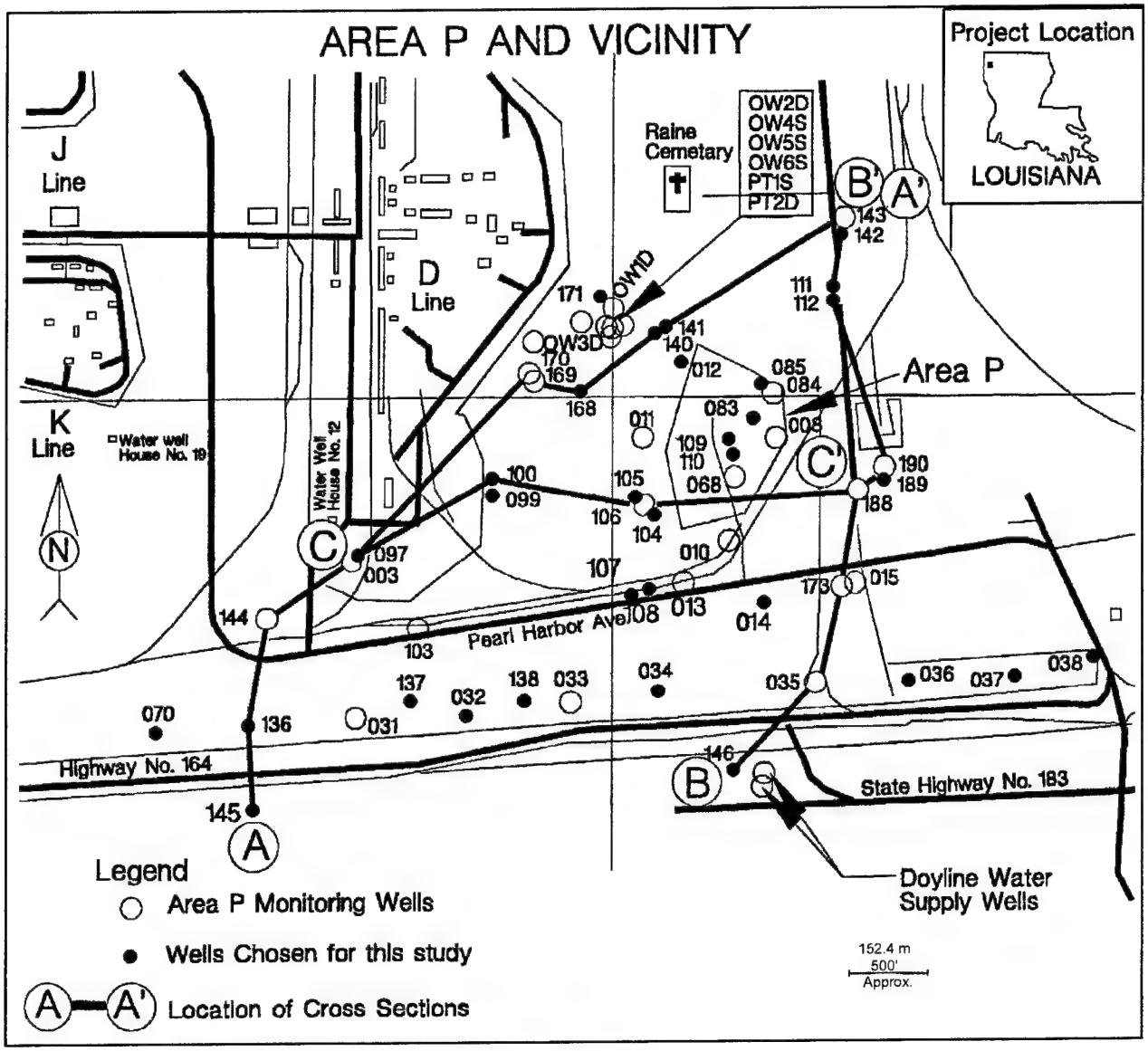

Figure 10. Geological transects at LAAP

Results of Groundwater Monitoring: JAAP

Explosives and their products in groundwater

Variability. The nine monthly sampling rounds exhibited limited variability for each analyte (Table 13), with three exceptions, Round 9 (January 1998) for MW131 and MW173 and Round 8 (December 1997) for WES1. If the data from these exceptional rounds are omitted, the variability (standard deviation) for TNB, TNT, 4ADNT, and 2ADNT averaged 12 to 17 percent of the mean. Variability for RDX and DNA, both of which exhibit small means (<10 ppb), was higher.

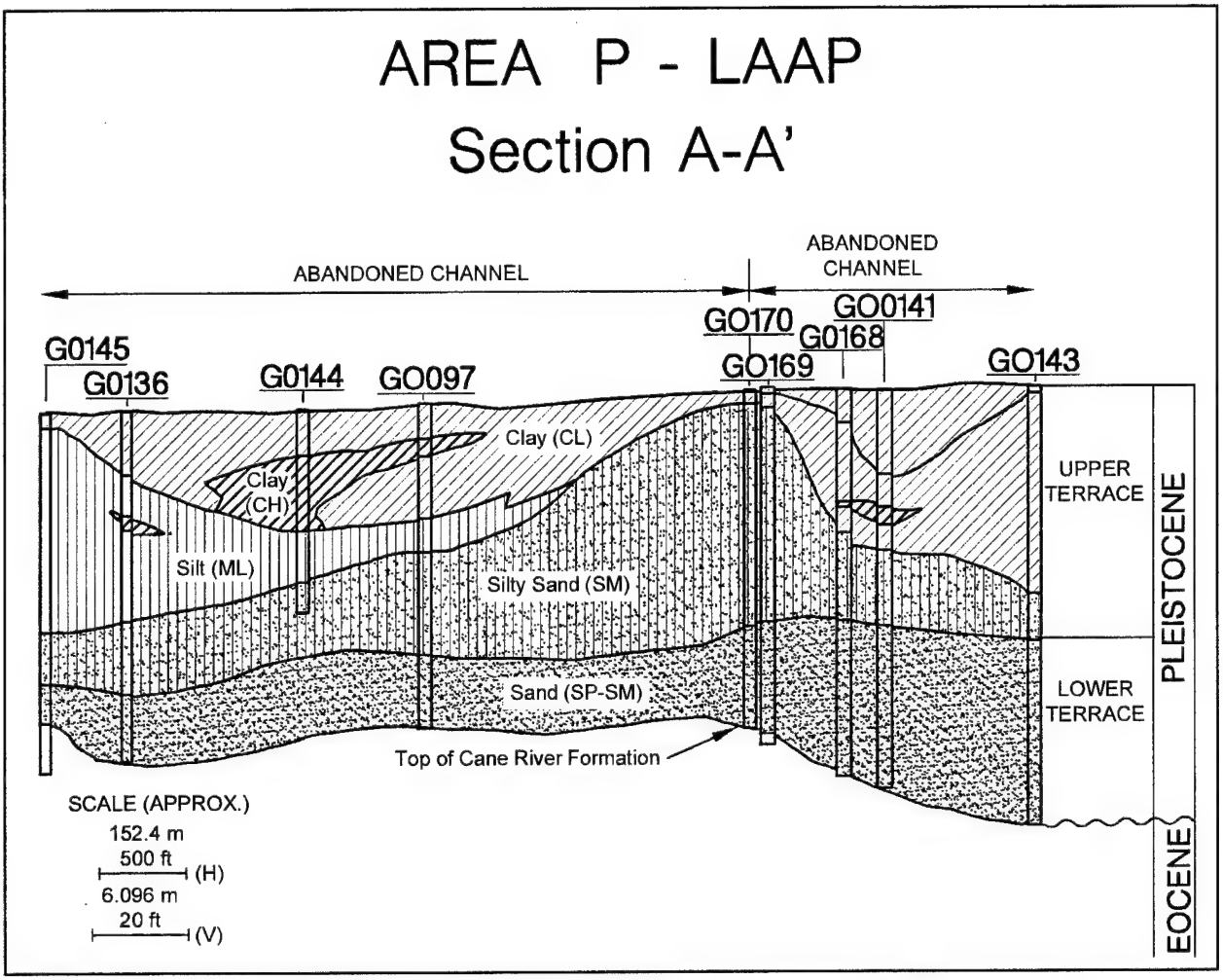

Figure 11. Geological Cross Section A-A' at LAAP

Trends in 1997-1998 data. Only five wells exhibited explosives in all sampling rounds; MW131, MW172, and MW173 in nine sampling rounds and new wells WES1 and WES3 in all six sampling rounds (Table 14, Figure 15). Four other wells, MW174, MW177, MW401, and WES2 showed one to three low-level sporadic detections. The most highly contaminated wells were MW131, MW172, MW173, WES1, and WES3. Two wells, MW175 and MW400, never exhibited explosives. MW175 was not sampled after Round 4 because of insufficient water level. MW400 was sampled in Rounds 1 through 5, dropped because of no detectable explosives, and sampled again in Round 9 when no explosives were found. MW176 was not sampled because of low water level.

The following analytes were never detected: tetryl, NB, 2,6DNT, 2,4DANT, 2,6DANT, 44'AZOXY, MNX, DNX, and TNX. The following analytes were detected rarely: 2,4DNT and picric acid in MW131 only, HMX (MW172 and MW173), and DNB (MW131 and WES1). Concentrations of picric acid were 233 and 260 $\mu g\ L^{-1}$ in MW131 in Rounds 1 and 3, respectively. A value of

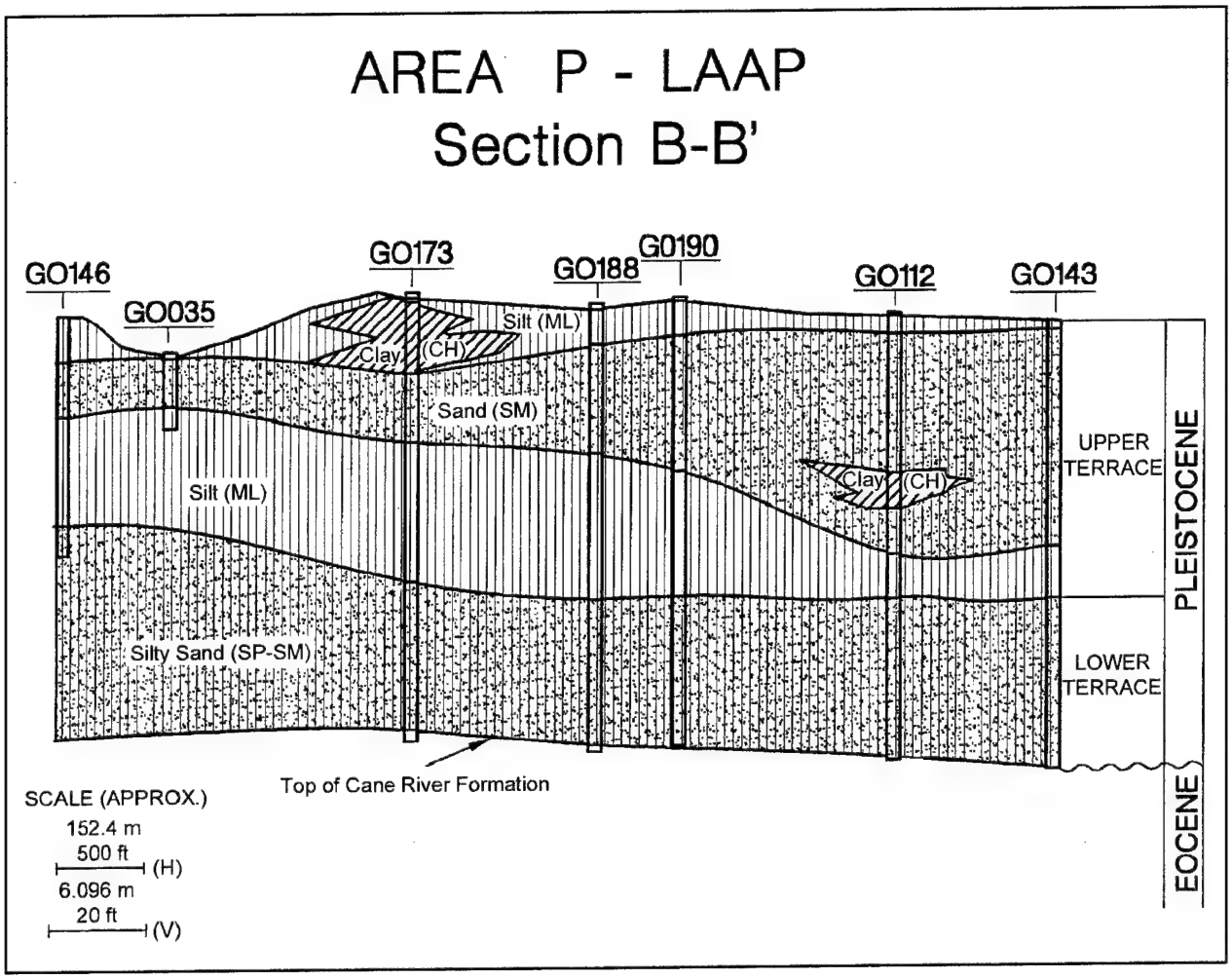

Figure 12. Geological Cross Section B-B' at LAAP

81 μg L^{-1} was reported for WES1. This value is below the statistically valid detection limit of 140 μg L^{-1}; therefore, the value is considered unreliable. The most commonly detected analytes were as follows:

Analyte	Number of Detections	Number of Wells
RDX	44	8
TNB	40	6
TNT	40	6
2ADNT	39	5
4ADNT	39	5
DNA	35	5
HMX	14	2
DNB	8	2
2,4DNT	3	1
Picric acid	2	1

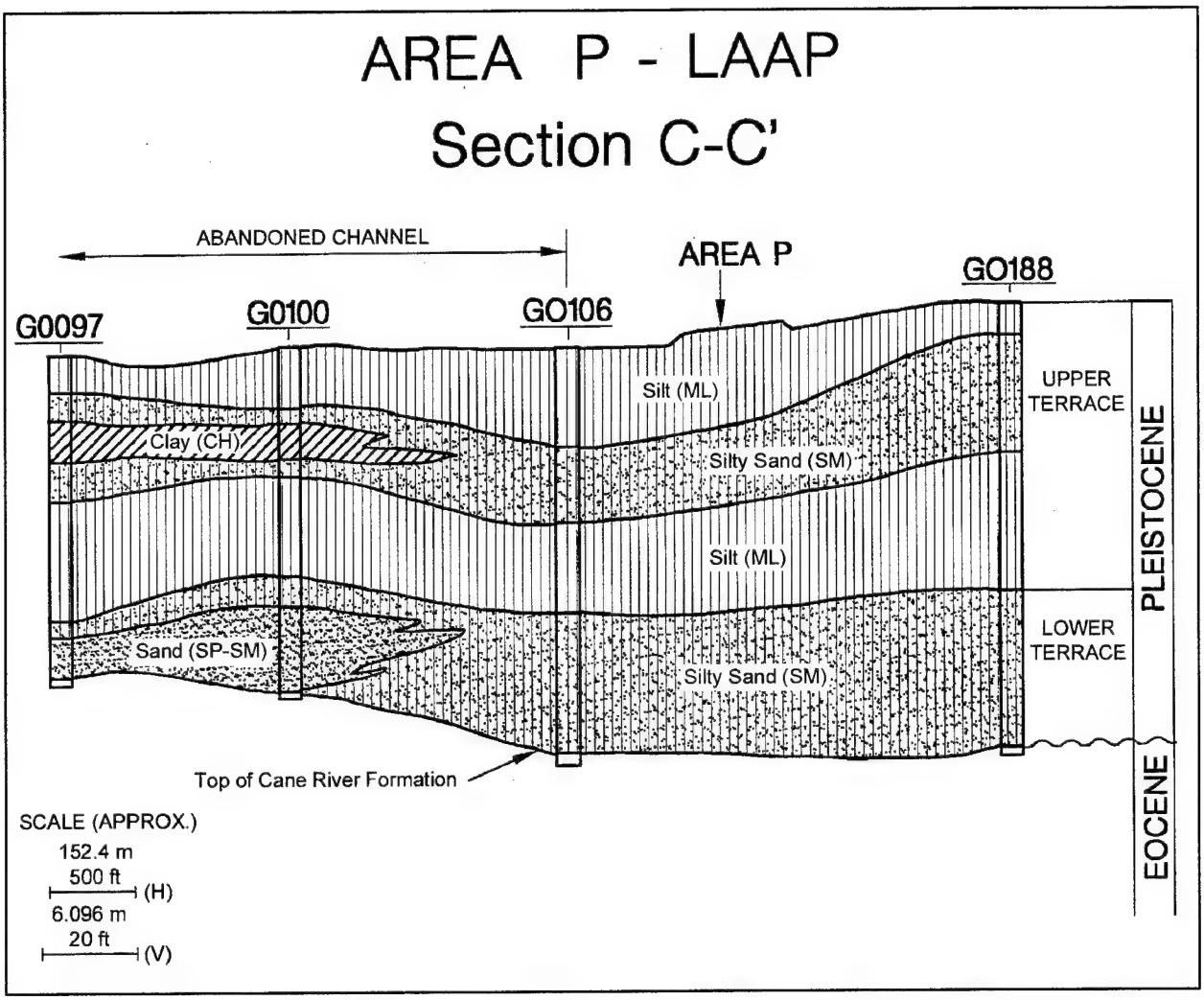

Figure 13. Geological Cross Section C-C' at LAAP

When the 1997-1998 data were regressed against time, data from only three wells exhibited linear regression coefficients (r^2) greater than 0.75, MW173, MW131, and WES3 (Table 14). In MW173, 4ADNT, 2ADNT, and HMX exhibited positive slopes of 0.36, 0.33, and 0.071, and r^2 values of 0.79, 0.79, and 0.85, respectively. In WES3, 2ADNT, 4ADNT, TNB, and TNT exhibited negative slopes with r^2 values less than 0.75. Except for the dramatic concentration increases observed for MW131 in Round 9, these results suggest that concentrations are changing too slowly to evaluate over a 9-month sampling period or that concentrations are no longer changing significantly at the site. Therefore, evaluating trends in concentration over time and changes in contaminant mass requires data that have been compiled over at least several years.

Trends in all available groundwater data. Available historical data were combined with 1997-1998 contaminant concentration data (omitting Round 9, January 1998) for trend analysis (Figure 16). Significant trends were observed for MW131, MW172, MW173, and WES3 (Table 15).

Table 12
Concentrations ($\mu g\ kg^{-1}$) of Explosives and Explosives Degradation Products in Soils Collected by CPT from Louisiana Army Ammunition Plant

Sample	TNT	RDX	HMX	2,4DNT	4ADNT	2ADNT	2,4DANT	2,6DANT	TNB	DNB	MNX
NA1-1U-9.1	81J[1]	206	200DL[2]	100DL	100DL	100DL	1,000DL	500DL	100DL	100DL	100DL
NA1-1U-11.9	98J	100DL	200DL	100DL	100DL	100DL	1,000DL	500DL	100DL	100DL	100DL
NA1-4U-7.8	40J	84J	200DL	100DL	100DL	100DL	1,000DL	500DL	100DL	100DL	100DL
NA2-1U-7.8	1,840	3,080	406	100DL	100DL	100DL	1,000DL	500DL	531	100DL	100DL
NA2-1U-10.2	1,140	3,910	990	100DL	100DL	83J	1,000DL	500DL	4,870	64J	100DL
NA2-1L-14.4	100DL	100DL	200DL	100DL	100DL	100DL	1,000DL	500DL	258	100DL	100DL
NA2-1L-18.9	1,660	1,690	53J	143	100DL	100DL	1,000DL	500DL	1,750	67J	100DL
NA2-3AU-5.5	100DL	100DL	200DL	100DL	100DL	100DL	1,000DL	500DL	39J	100DL	100DL
NA2-3L-17.4	219	308	200DL	46J	100DL	100DL	1,000DL	500DL	89	100DL	100DL
NA4-2VP-3.1	208	1,080	92J	100DL	25J	25J	1,000DL	500DL	100DL	100DL	100DL
NA4-2VP-4.6	2,570	3,930	328	47J	33J	100DL	1,000DL	500DL	84J	86J	100DL
NA4-2U-5.4	2,730	4,860	479	78J	231	409	116J	47J	47J	94J	100DL
NA4-2VP-7.7	7,060	8,380	531	302	100DL	100DL	1,000DL	500DL	274	195	100DL
NA4-4L-20.2	1,680	843	200DL	166	100DL	100DL	1,000DL	500DL	1,390	53J	100DL
NA4-5VP-3.1	312	882	132	100DL	100DL	26J	1,000DL	500DL	155	31J	100DL
NA4-5VP-4.6	4,990	6,120	428	100DL	100DL	88J	1,000DL	500DL	2,280	139	100DL
NA4-5VP-6.1	6,510	4,070	325	316	248	257	1,000DL	500DL	1,310	124	101
NA4-5VP-7.6	5,470	11,800	1,390	100DL	100DL	100DL	1,000DL	500DL	13,100	209	120
NA4-5VP-9.4	1,970	9,750	1,810	100DL	100DL	144	1,000DL	500DL	12,000	72J	100DL
NA4-5VP-10.7	1,060	6,860	1,540	100DL	100DL	100DL	1,000DL	500DL	7,840	32J	98J
NA4-5VP-16.8	100DL	392	65J	100DL	100DL	100DL	1,000DL	500DL	385	53J	100DL
NA4-5VP-18.3	100DL	2,170	375	100DL	100DL	100DL	1,000DL	500DL	1,300	269	240
NA4-5VP-20.5	1,380	1,990	99J	130	100DL	100DL	1,000DL	500DL	1,110	63J	100DL
NA6-1U-11.8	940	82J	56J	100DL	100DL	100DL	1,000DL	500DL	3,010	100DL	100DL
NA6-1U-14.9	100DL	209	200DL	100DL	100DL	100DL	1,000DL	500DL	115	100DL	100DL
NA6-2VP-4.6	650	544	29J	100DL	100DL	100DL	1,000DL	500DL	100DL	100DL	100DL
NA6-2VP-6.1	1,320	386	66J	100DL	25J	106	1,000DL	500DL	1,400	100DL	100DL

(Continued)

[1] J values are below the statistically reliable detection limits.
[2] Detection limit.

Table 12 (Concluded)

Sample	TNT	RDX	HMX	2,4DNT	4ADNT	2ADNT	2,4DANT	2,6DANT	TNB	DNB	MNX
NA6-2VP-7.6	3,050	789	376	100DL	100DL	100DL	1,000DL	500DL	5,750	100DL	100DL
NA6-2VP-8.2	5,120	4,360	1,060	100DL	100DL	541	1,000DL	500DL	7,710	100DL	100DL
NA6-2VP-9.1	3,900	5,750	1,310	100DL	100DL	100DL	1,000DL	500DL	9,230	100DL	100DL
NA6-2VP-10.4	2,170	1,810	482	100DL	100DL	100DL	1,000DL	500DL	6,510	100DL	100DL
NA6-2VP-12.3	4,750	7,100	471	100DL	100DL	152	1,000DL	500DL	8,630	100DL	100DL
NA6-2VP-13.1	1,130	2,480	56J	100DL	100DL	100DL	1,000DL	500DL	1,600	100DL	100DL
NA6-2VP-13.7	48J	44J	200DL	100DL	100DL	100DL	1,000DL	500DL	88J	100DL	100DL
NA6-2VP-15.2	274	620	200DL	100DL	100DL	100DL	1,000DL	500DL	389	100DL	100DL
NA6-2VP-17.5	106	515	200DL	100DL	100DL	100DL	1,000DL	500DL	234	100DL	100DL
NA6-2VP-18.3	100DL	571	200DL	100DL	100DL	100DL	1,000DL	500DL	106	100DL	100DL
NA6-3U-6.3	372	729	95J	100DL	100DL	100DL	1,000DL	500DL	100DL	100DL	100DL
NA6-3-10.5	1,980	2,530	599	100DL	100DL	100DL	1,000DL	500DL	566	28J	100DL
NA6-3-13.1	905	3,590	149	100DL	100DL	100DL	1,000DL	500DL	538	33J	100DL
NA6-3-15.9	649	3,390	91J	100DL	100DL	100DL	59J	500DL	288	27J	100DL
NA6-3-17.9	763	3,610	82J	100DL	100DL	100DL	96J	500DL	353	33J	100DL
NA7-1-5.0	100DL	108	75J	100DL	100DL	100DL	1,000DL	500DL	100DL	100DL	100DL
NA7-2VP-1.6	71J	174	426	100DL	100DL	42J	1,000DL	500DL	446	100DL	100DL
NA7-2VP-3.1	634	1,240	1,830	100DL	100DL	100DL	1,000DL	500DL	7,490	100DL	100DL
NA7-2VP-4.6	670	1,720	879	100DL	40J	119	1,000DL	500DL	3,270	100DL	100DL
NA7-2VP-6.3	583	409	160J	100DL	100DL	80J	100DL	500DL	2,200	100DL	100DL
NA7-2VP-7.6	1,560	2,930	313	100DL	100DL	100DL	78J	500DL	8,570	100DL	100DL
NA7-2VP-9.1	320	987	200DL	100DL	100DL	100DL	26J	500DL	1,230	100DL	100DL
NA7-2VP-10.6	100DL	212	200DL	100DL	100DL	100DL	1,000DL	500DL	39J	100DL	100DL
NA7-2VP-16.8	100DL	47J	200DL	100DL	100DL	100DL	1,000DL	500DL	82J	100DL	100DL

TNT concentrations were decreasing in two of the wells, MW172 and WES3. RDX was decreasing in MW172. TNB was decreasing in MW173 and WES1, but increasing in MW131. Except for declines in WES3, significant trends for the TNT transformation products 2ADNT, 4ADNT, and DNA were increasing. The concentrations of 2,4DNT were decreasing in MW131.

As observed with the historical data at LAAP, the historical data for JAAP were limited and highly variable (Table 15). Of the 216 statistical tests (12 wells × 18 analytes), only about 25 percent had values above detection limits. Of these, about 20 percent showed significant decreases in concentration over time, while

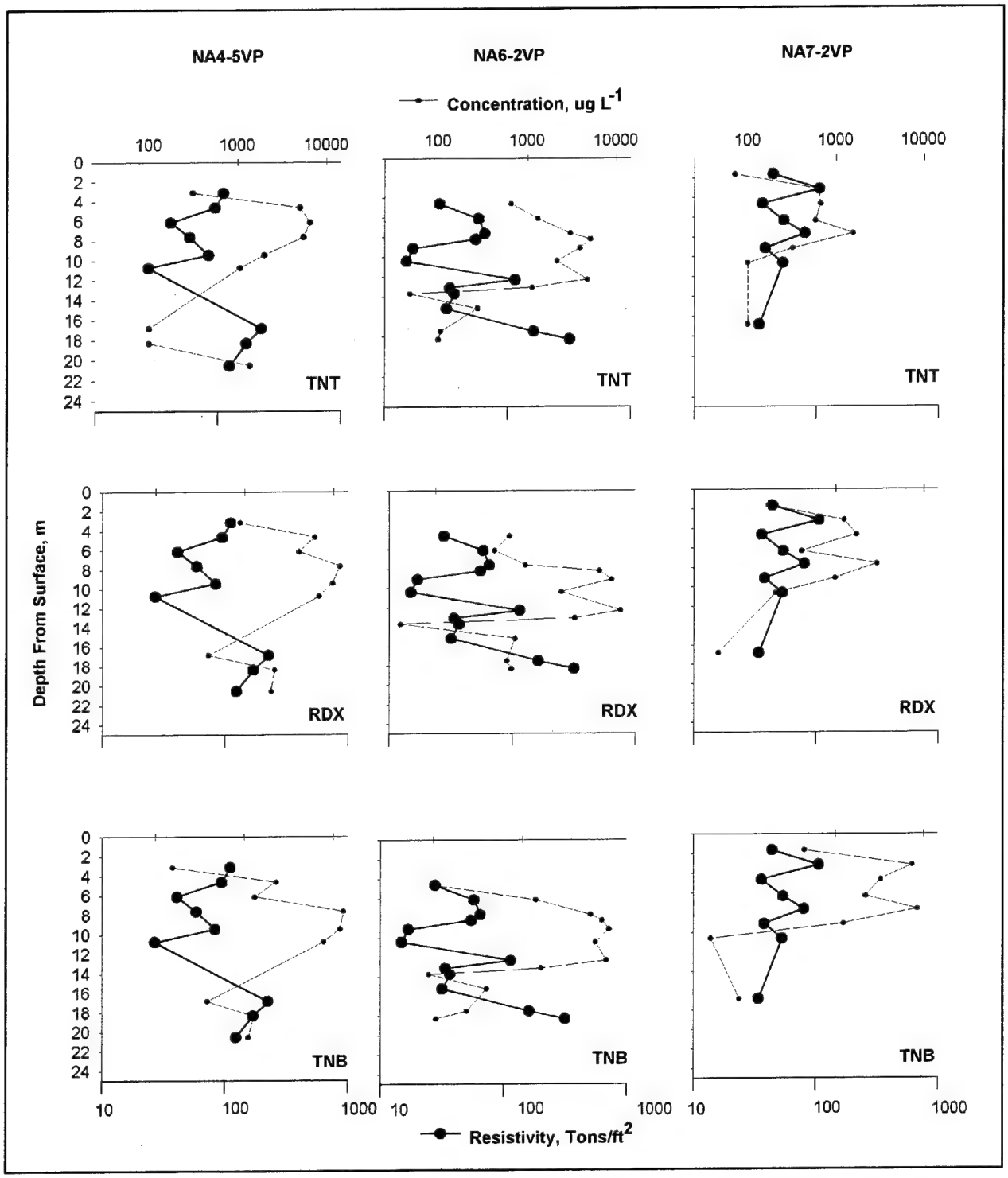

Figure 14. Concentrations of explosives (TNT, RDX, and TNB) and CPT tip resistivities with depth at three LAAP CPT locations

Table 13
Groundwater Concentrations ($\mu g\ L^{-1}$) of Explosives and Degradation Products for Site L1, JAAP

Well No.	Date	TNB	DNB	TNT	4ADNT	2ADNT	HMX	RDX	3,5DNA
MW131	05/17/97	1,420	0.2DL[1]	1,290	28.2	40.5	0.2DL	3.11	11.8
	06/12/97	2,000	2.79	1,740	39.8	57.1	0.2DL	3.78	10.6
	07/11/97	1,440	0.2DL	1,160	28.8	41.9	0.2DL	4.27	0.2DL
	08/09/97	1,930	1.86	1,540	36.7	55.2	0.2DL	4.10	7.7
	09/06/97	1,870	2.18	1,510	36.3	56.1	0.2DL	2.96	15.7
	10/02/97	1,910	2.17	1,580	38.2	57.6	0.2DL	2.80	6.8
	10/31/97	1,930	1.60	1,590	38.2	58.5	0.2DL	2.46	4.9
	12/02/97	1,920	1.81	1,630	38.5	62.5	0.2DL	2.62	5.6
	01/12/98	2,950	3.40	6,830	67.4	87.6	0.2DL	2.18	39.8
	03/17/98	3,630	7.02	12,130	91.8	93.3	0.2DL	3.59	89.7
MW172	05/16/97	2.13	0.2DL	9.09	2.58	1.99	0.40	5.01	0.32
	06/12/97	2.28	0.2DL	9.17	2.65	2.09	0.2DL	5.00	0.23
	07/10/97	2.01	0.2DL	8.56	2.53	2.00	0.2DL	4.65	0.2DL
	08/06/97	1.79	0.2DL	7.68	2.54	1.99	0.25	3.96	0.2DL
	09/05/97	1.99	0.2DL	8.49	2.81	2.19	0.31	4.11	0.32
	10/02/97	1.72	0.2DL	7.51	2.78	2.14	0.2DL	3.65	0.34
	10/30/97	1.79	0.2DL	8.05	2.88	2.20	0.2DL	3.77	0.45
	12/04/97	1.94	0.2DL	8.87	3.17	2.49	0.28	4.76	0.54
	01/15/98	1.85	0.2DL	7.92	2.59	2.14	0.29	4.18	0.52
	03/18/98	2.35	0.2DL	9.76	2.56	2.02	0.2DL	5.85	0.58
MW173	05/17/97	3.43	0.2DL	25.1	5.19	5.70	1.58	16.6	0.68
	06/12/97	4.57	0.2DL	31.4	5.09	5.78	1.71	19.4	0.44
	07/10/97	5.27	0.2DL	32.6	5.66	6.11	1.66	22.2	0.2DL
	08/07/97	5.44	0.2DL	34.6	6.53	7.07	1.85	22.0	0.32
	09/05/97	5.16	0.2DL	33.8	6.97	7.35	1.76	20.7	0.32
	10/02/97	4.80	0.2DL	33.2	7.10	7.33	1.78	21.1	0.53
	10/31/97	5.80	0.2DL	35.9	7.87	8.22	2.04	22.7	0.66
	12/05/97	4.76	0.2DL	32.8	6.86	7.32	2.17	22.7	0.71

(Continued)

[1] Detection limit.
[2] J values are below the statistically reliable detection limits.

Table 13 (Concluded)

Well No.	Date	TNB	DNB	TNT	4ADNT	2ADNT	HMX	RDX	3,5DNA
MW173 (Cont.)	01/15/97	0.30	0.2DL	4.59	3.33	3.61	0.81	8.19	0.47
	03/18/98	3.28	0.2DL	19.1	4.41	4.63	1.24	12.8	0.61
WES1	08/07/97	12.2	0.2DL	12.2	4.69	6.27	0.2DL	0.63	4.31
	09/06/97	10.5	0.2DL	11.7	8.34	6.70	0.2DL	1.89	6.69
	10/02/97	12.7	0.21	16.5	11.5	8.37	0.2DL	0.26	8.80
	10/31/97	6.8	0.2DL	10.5	7.13	6.63	0.2DL	0.2DL	7.75
	12/05/97	2.4	0.2DL	4.7	3.80	4.12	0.2DL	0.41	4.09
	01/15/98	10.6	0.2DL	14.7	6.85	7.51	0.2DL	1.23	8.45
	03/18/98	30.1	0.13J[2]	35.6	12.3	13.5	0.2DL	0.58	15.7
WES3	08/07/97	0.99	0.2DL	1.69	0.87	0.84	0.2DL	1.30	0.21
	09/05/97	0.56	0.2DL	1.23	0.93	0.72	0.2DL	0.75	0.36
	10/01/97	0.56	0.2DL	1.07	0.88	0.67	0.2DL	0.68	0.38
	10/31/97	0.52	0.2DL	0.95	0.85	0.62	0.2DL	0.59	0.45
	12/04/97	0.53	0.2DL	0.93	0.75	0.63	0.2DL	0.67	0.41
	01/14/98	0.72	0.2DL	0.99	0.65	0.57	0.2DL	0.65	0.38

about 11 percent showed increases, and the remainder showed no trends (Table 16). A closer examination of the results reveals that all of the increases occurred for the transformation products of TNT, 2ADNT and 4ADNT, or for TNB and its transformation product DNA (Table 15), until the Round 9 anomalies (see below). The number of wells exhibiting analyte decreases were small, but were in the relative order of decreasing analytes TNT=TNB>RDX=HMX= 4ADNT=2ADNT=26DNT.

Round 9 anomalies and extra sampling (Round 10). Concentrations of most detected analytes increased dramatically in MW131 in the Round 9 (January 1998) sampling (Table 13). Concentrations of TNT increased by a factor of six over previous rounds. The 3,5DNA increased by a factor of eight; TNB, 4ADNT, and DNB increased by a factor of two; and 2ADNT increased by a factor of nearly two. RDX remained within the previous range. Concentrations of TNT, 3,5DNA, 4ADNT, 2ADNT, HMX, and TNB in MW173 dropped significantly.

Results from a follow-up additional sampling (March 1998) of MW131, MW172, MW173, WES1, and WES2 revealed even higher contaminant concentrations in MW131 (Table 13). The concentration of TNT was twice that of

Table 14
Regression Statistics for Contaminant Concentration Trend Analyses, Rounds 1-8, JAAP

Explosive	Well	MLE Regression Statistics[1]		OLS Regression Statistics[2]				Trend (Slope)
		X^2	P	Model	F	P	r^2	
2,6DNT	MW131	4.726	0.0297 *[3]	Linear	17.623	0.0018 *	0.638	-
2ADNT	MW131	11.021	0.0009 *	Linear	8.266	0.0282 *	0.579	+
	MW172	15.789	0.0001 *	Linear	11.842	0.0138 *	0.664	+
	MW173	31.382	0.0001 *	Linear	23.537	0.0028 *	0.797	+
	WES3	26.614	0.0001 *	Linear	15.969	0.0281 *	0.842	-
DNA	MW172	8.733	0.0031 *	Linear	6.550	0.0430 *	0.522	+
4ADNT	MW172	20.432	0.0001 *	Linear	15.324	0.0079 *	0.719	+
	MW173	30.279	0.0001 *	Linear	22.709	0.0031 *	0.791	+
	WES3	7.033	0.0080 *	Quadratic	19.577	0.0486 *	0.951	-
HMX	MW173	787.734	0.0001 *	Linear	612.682	0.0001 *	0.989	-
RDX	MW131	0.018	0.8933	Quadratic	105.335	0.0001 *	0.963	NS[4]
	MW172	32.566	0.0001 *	Linear	26.645	0.0006 *	0.748	-
	MW173	2.805	0.0940	Linear	2.295	0.1641	0.203	NS
	WES1	1.593	0.2069	Linear	0.961	0.3992	0.243	NS
	WES2	2.870	0.0902	Linear	1.722	0.2808	0.365	NS
	WES3	7.986	0.0047 *	Linear	4.792	0.1164	0.615	NS
TNB	MW131	10.385	0.0013 *	Linear	8.654	0.0147 *	0.464	+
	MW172	5.106	0.0238 *	Quadratic	5.019	0.0309 *	0.501	-
	MW173	0.297	0.5859	Linear	0.274	0.6108	0.024	NS
	WES1	13.422	0.0002 *	Linear	8.053	0.0658	0.729	NS
	WES3	17.769	0.0001 *	Linear	10.661	0.0469 *	0.780	-
TNT	MW131	0.998	0.3178	Linear	0.832	0.3832	0.077	NS
	MW172	7.929	0.0049 *	Linear	6.710	0.0251 *	0.379	-
	MW173	5.429	0.0198 *	Linear	4.594	0.0553	0.295	NS
	WES1	2.874	0.0900	Linear	1.724	0.2805	0.365	NS
	WES3	24.575	0.0001 *	Linear	14.745	0.0312 *	0.831	-

[1] Maximum likelihood estimate (MLE) regression statistic basic on a normal distribution.
[2] Ordinary least squares regression statistic.
[3] * = Statistically significant; $\alpha = 0.05$.
[4] Trend not considered significant.

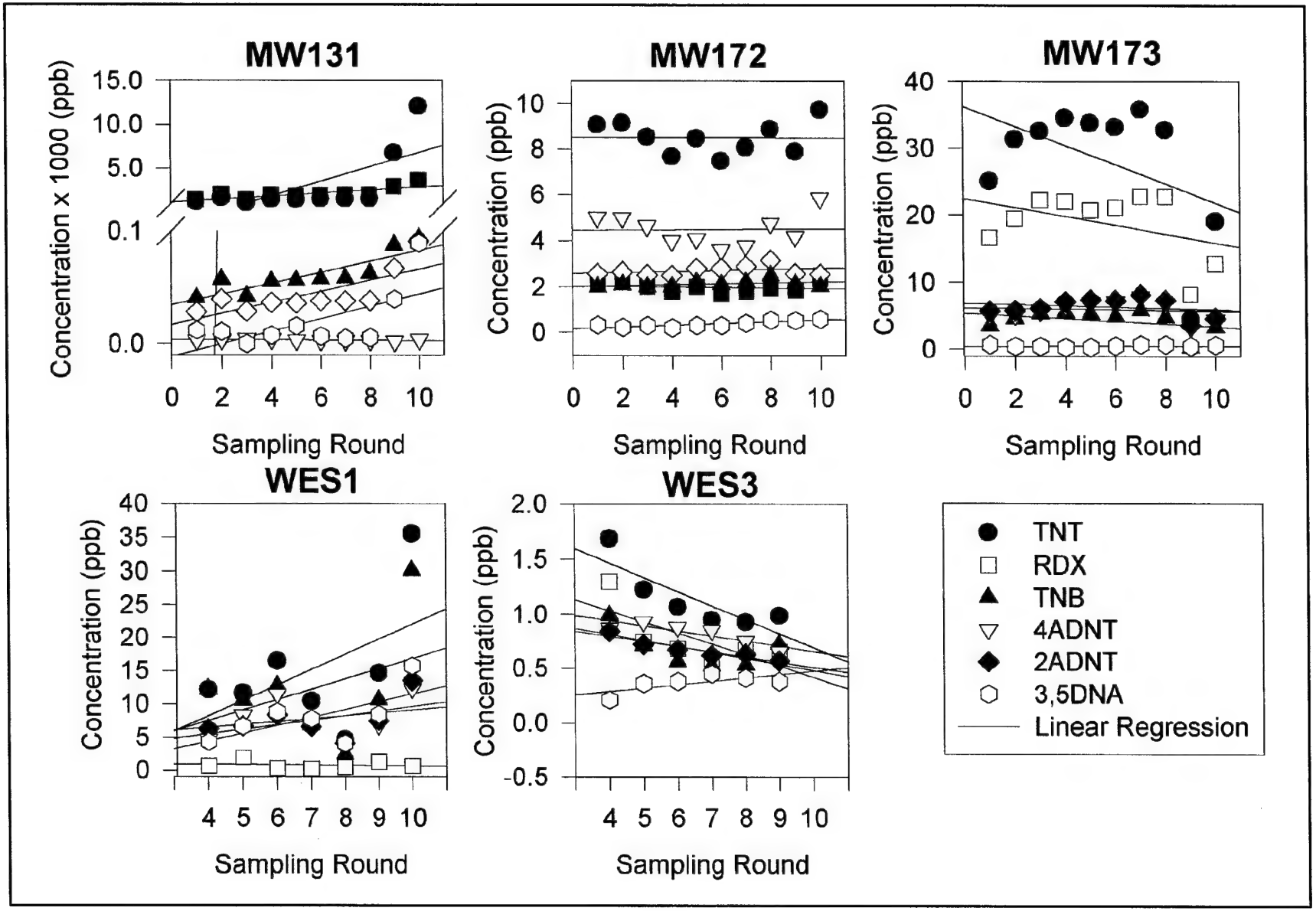

Figure 15. Trends in explosives concentration over sampling events (1997-1998) at JAAP

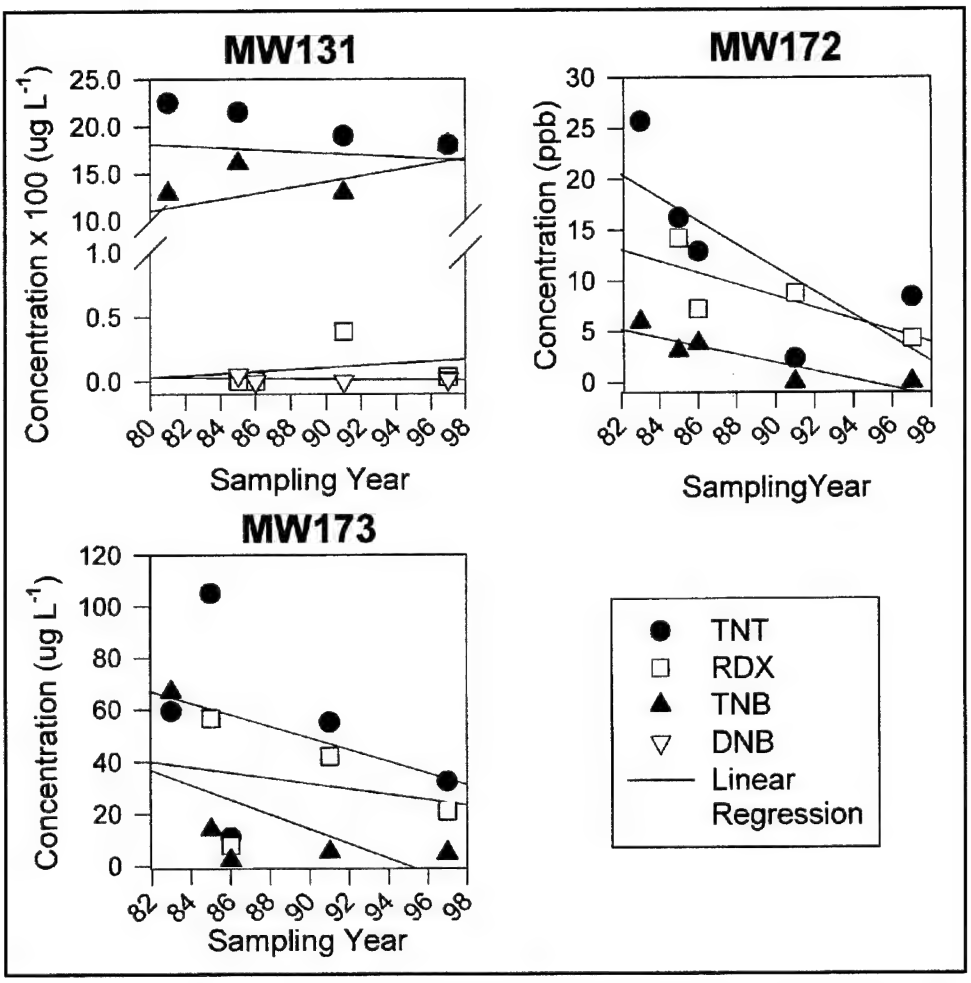

Figure 16. Trends in explosives concentrations over sampling events (1982-1997) at JAAP

Round 9. Concentrations in MW173 had increased, but remained lower than observed in Rounds 1-8. Round 10 samples also showed the appearance of several analytes not observed above detection limits in the first nine rounds: 2,6DANT in MW131 (9.55 μg L^{-1}) and in WES2 (6.67 μg L^{-1}); 2,4DANT in MW131 (4.01 μg L^{-1}) and in WES2 (5.27 μg L^{-1}); and 2,4DNT in WES2 (0.52 μg L^{-1}).

Results from the confirmation laboratory (U.S. Army Cold Regions Research and Engineering Laboratory) for Round 9 (MW131, MW172, MW173, and WES1) and Round 10 (MW131, MW172, MW173, WES1, and WES2) were within 10 percent of values determined by the Environmental Chemistry Branch, WES.

Table 15
Statistically Significant Trends in Contaminant Concentration Data from JAAP Wells, 1981-1997

Well	Contaminant	Trend	N	Remarks
MW131	2,6DNT	Decreasing	12	Increasing from 3 ppb in 1981 to 8 ppb in 1986, then dropping to <0.2 ppb from 1991 on
	2ADNT	Increasing	8	No historical data; generally increasing from 40 ppb in May 1997 to 63 ppb in Dec 1997
	TNB	Increasing	12	Wide range of concentrations from 755 to 2,000 ppb over the whole time period; considerable variability
MW172	2ADNT	Increasing	8	No historical data; 1997 concentrations all in the range from 2.0 to 2.5 ppb
	DNA	Increasing	8	No historical data; 1997 concentrations all in the range from 0.17 to 0.54 ppb
	4ADNT	Increasing	8	No historical data; 1997 concentrations all in the range from 2.53 to 3.17 ppb
	RDX	Decreasing	11	General decrease from 14 ppb in 1985 to 3-5 ppb in 1997
	TNB	Decreasing	13	Historical data highly variable; maximum 9.2 ppb in 1983, declining to <2 ppb in 1997
	TNT	Decreasing	13	Historical data highly variable; maximum 41 ppb in 1983, declining to 7-9 ppb in 1997
MW173	2ADNT	Increasing	8	No historical data; 1997 concentrations all in the range from 5.70 to 8.22 ppb
	4ADNT	Increasing	8	No historical data; 1997 concentrations all in the range from 5.09 to 7.87 ppb
	HMX	Decreasing	9	Only one historical data point, 44 ppb in 1991; 1997-98 concentrations 1-2 ppb
WES3	2ADNT	Decreasing	5	No historical data; slight decline from 0.84 ppb in Aug 1997 to 0.63 ppb in Dec 1997
	4ADNT	Decreasing	5	No historical data; slight increase initially, then decline from 0.93 ppb in Sep 1997 to 0.75 ppb in Dec 1997
	TNB	Decreasing	5	No historical data; slight decline from 0.99 ppb in Aug 1997 to 0.53 ppb in Dec 1997
	TNT	Decreasing	5	No historical data; slight decline from 1.69 ppb in Aug 1997 to <1 ppb in Dec 1997

Geochemical parameters in groundwater

General groundwater quality. Concentrations of calcium and magnesium suggest that JAAP groundwater is rather hard and may require softening (when >200 mg L^{-1}) if ever put to household use (Table 17). Manganese concentrations were generally low, but several values (MW131, MW177, MW400, and WES1) were in exceedance of the drinking water regulatory limit of 0.05 mg L^{-1}. Four

Table 16
Trend Summary for JAAP Groundwater: Number of Wells out of 12 Wells Sampled, Rounds 1-8

Contaminant	Wells with <3 Detectable Concentrations	Probable Trend		
		Not Significant	Decreasing	Increasing
2,4DANT	12			
2,4DNT	11	1		
2,6DANT	12			
2,6DNT	11		1	
2ADNT	7	1	1	3
DNA	7	4		1
44'AZOXY	12			
4ADNT	7	2	1	2
DNB	11	1		
DNX	12			
HMX	10	1	1	
MNX	12			
NB	12			
RDX	6	5	1	
Tetryl	12			
TNB	7	2	2	1
TNT	7	3	2	
TNX	12			

wells (MW131, MW175, MW400, and MW401) exhibited total iron concentrations above the drinking water standard of 0.3 mg L^{-1}. Values for all wells ranged from less than the detection limit of 0.02 to 1.78 mg L^{-1} (MW401). Nitrate/nitrite nitrogen values were generally low. The range for natural waters is 0.1 to 10 mg L^{-1} (Driscoll 1986). Most values were much less than 10 mg L^{-1}. Values in the range of 20 to 90 mg L^{-1} in drinking water are considered harmful to infants. Values for two samples taken from MW131 fell within the lower end of this limit; 24.9 and 26.6 mg L^{-1} collected on October 2 and 10, 1997, respectively. Sulfate concentrations were relatively low. Most values were less than 100 mg L^{-1}. Chloride concentrations less than 150 mg L^{-1} are considered satisfactory for most purposes. Chloride concentrations were only slightly higher than this value. No methane or reduced iron was observed.

Table 17
Concentrations of Geochemical Parameters in JAAP Groundwater (mg L^{-1})

Well No.	Date	Calcium	Magnesium	Manganese	Total Iron	Nitrate/Nitrite Nitrogen	Sulfate	Chloride
MW131	05/17/97	108	47.7	0.058	0.195	4.58	53.6	5.28
	06/12/97	104	44.6	0.018	<0.02	4.91	48.9	4.74
	07/11/97	99.9	45.7	0.012	<0.02	9.06	64.2	4.82
	08/09/97	125	50.5	0.027	<0.02	7.93	34.7	2.49
	09/06/97	165	60.8	0.071	0.033	8.54	5.18	<0.38
	10/02/97	135	62.9	0.133	0.123	24.9	3.92	<0.38
	10/31/97	155	79.6	1.02	2.05	26.6	118	6.94
	12/02/97	161	68	0.467	0.823	6.01	108	9.67
	01/12/98	127	49.2	0.03	0.081	5.81	62.5	2.9
MW172	05/16/97	94.6	45.1	<0.001	0.026	3.15	62.4	11.4
	06/12/97	93	44.2	<0.001	<0.02	3.54	57.5	10.8
	07/10/97	90.9	45.4	<0.001	<0.02	4.08	62.2	11.1
	08/06/97	101	44.8	<0.001	<0.02	4.37	30.6	5.8
	09/05/97	117	44.6	<0.001	<0.02	4.02	2.95	0.435
	10/02/97	93.8	47.8	<0.001	<0.02	3.72	2.49	0.391
	10/30/97	96.8	47.8	<0.001	0.045	8.09	73.5	9.06
	12/04/97	106	47.5	<0.001	<0.02	2.94	68.7	8.82
	01/15/98	105	48.1	<0.001	<0.02	2.45	70.3	7.72
MW173	05/17/97	130	59.6	0.004	<0.02	2.37	191	6.83
	06/12/97	101	47.8	0.002	<0.02	2.5	86.8	7.11
	07/10/97	96.5	44.1	0.006	0.058	2.16	79.2	8
	08/07/97	91.6	44.3	<0.001	<0.02	2.61	29.1	3.61
	09/05/97	107	42.8	<0.001	<0.02	2.82	2.91	<0.38
	10/02/97	95.8	46.3	0.003	<0.02	2.3	0.522	<0.38
	10/31/97	89.8	45.4	0.022	0.134	3.06	65.1	7.23
	12/05/97	105	46.7	0.009	0.021	2.39	66.4	7.84
	01/15/98	401	214	0.022	0.049	1.65	1,433	15.8
MW174	05/16/97	89.03	42	0.002	0.026	<0.02	54.5	8.93

(Sheet 1 of 4)

[1] NS = Not assayed.

Table 17 (Continued)

Well No.	Date	Calcium	Magnesium	Manganese	Total Iron	Nitrate/Nitrite Nitrogen	Sulfate	Chloride
MW174 (Cont.)	06/11/97	92.24	39.9	<0.001	<0.025	<0.02	51.2	9.84
	07/09/97	83.3	NS[1]	<0.001	0.026	<0.02	58.1	8.34
	08/06/97	89.6	41.8	<0.001	0.025	<0.02	27.1	4.41
	09/04/97	113	39.7	<0.001	0.052	<0.02	3.04	0.512
	10/01/97	97	44.6	0.001	0.036	0.177	2.7	0.742
	10/29/97	96.1	47.2	0.004	0.03	0.262	83.1	27.8
	12/03/97	108	48.3	0.009	0.04	0.025	7.8	6.12
	01/13/98	113	47	0.005	0.021	<0.02	89.9	38.9
MW175	05/15/97	75.2	33.8	<0.001	<0.02	0.28	43.9	1.16
	06/11/97	83.56	35.7	0.021	0.492	0.359	44.6	1.45
	07/09/97	128	NS	0.313	3.02	0.25	46.7	0.832
MW177	05/15/97	93.6	46.0	0.033	<0.02	0.13	79.3	3.34
	06/11/97	96.76	46.4	0.062	<0.02	0.132	89.6	3.51
	07/09/97	87.7	NS	0.066	<0.02	0.112	89.4	3.31
	08/05/97	92.9	46.0	0.088	<0.02	0.081	43.3	1.86
	09/04/97	117	48.2	0.110	<0.02	0.074	4.2	<0.375
	09/30/97	96.3	47.1	0.063	<0.02	0.051	3.22	<0.375
	10/29/97	96.2	50.0	0.062	<0.02	0.051	92.4	3.69
	12/03/97	95.5	46.4	0.064	<0.02	0.075	75.4	29.6
	01/13/98	96.1	45.1	0.026	<0.02	0.126	85.5	3.56
MW178	05/15/97	97.1	47.6	<0.001	<0.02	3.49	48.1	7.7
	06/11/97	100.3	48	<0.001	<0.02	3.75	55.8	7.88
	07/09/97	96.2	NS	<0.001	<0.02	4.68	62.3	9.31
	08/05/97	103.0	47.2	0.002	<0.02	5.4	30.2	5.35
	09/04/97	112.0	47.3	<0.001	0.021	5.63	3.04	0.478
	09/30/97	89.1	48.9	<0.001	<0.02	7.98	2.5	0.483
	10/28/97	89.8	49.5	<0.001	<0.02	8.83	69.4	13.5
	12/02/97	91.0	49.1	<0.001	<0.02	4.32	64.3	11.5
	01/13/98	102.0	49.7	<0.001	<0.02	3.78	61	8.87

(Sheet 2 of4)

Table 17 (Continued)

Sample	Date	Calcium	Magnesium	Manganese	Total Iron	Nitrate/Nitrite Nitrogen	Sulfate	Chloride
MW400	05/14/97	111	52	0.218	0.754	1.05	97.3	21.9
	06/10/97	109.9	51.1	0.277	0.755	<0.02	102	22.7
	07/08/97	106	NS	0.244	1.02	<0.02	105	22.2
	08/06/97	107	52.2	0.246	1.27	<0.02	51.2	10.9
	09/04/97	102	49.5	0.239	1.22	<0.02	5.31	0.93
	01/12/98	114	51	0.213	1.13	<0.02	108	22.3
MW401	05/16/97	110	53.1	0.027	1.49	<0.02	68.2	50.1
	06/11/97	109.2	52.9	0.025	1.61	<0.02	71.4	42.3
	07/09/97	102	51.6	0.023	1.51	<0.02	78.2	31.2
	08/06/97	110	49.4	0.024	1.77	<0.02	38.5	16
	09/05/97	128	48.3	0.024	1.78	<0.02	3.91	1.24
	10/01/97	99.2	52.2	0.033	1.57	0.263	3.14	1.17
	10/29/97	98.4	51.4	0.032	1.52	0.067	86.8	25
	12/03/97	110	51.9	0.038	1.31	<0.02	71.1	18.2
	01/14/98	110	52	0.027	1.6	<0.02	75.2	38.6
WES1	08/07/97	97.2	46.4	0.04	<0.02	5.19	35.6	4.17
	09/06/97	112	46.5	0.036	0.023	5.08	3.55	<0.38
	10/02/97	102	48.3	0.056	0.022	5.22	0.479	<0.38
	10/31/97	96.7	48.4	0.033	0.024	4.58	77.3	6.87
	12/05/97	101	46	0.013	<0.02	3.38	73.3	7.46
	01/15/98	96.7	43	0.017	<0.02	4.2	69.3	17.7
WES2	08/07/97	98.6	47.2	0.004	<0.02	2.61	33.1	2.75
	09/05/97	119	46.7	0.002	0.032	3.07	3.26	<0.38
	10/01/97	98.5	49.9	0.001	0.054	2.83	2.6	<0.38
	10/30/97	99.9	50.7	<0.001	0.024	3.38	72.4	5.59
	12/04/97	98.4	50.8	0.002	<0.02	1.6	31.1	3.42
	01/14/98	105	49.2	0.019	0.019	3.85	71.1	20.9
WES3	08/07/97	108	48.4	<0.001	<0.20	3.29	36.1	3.92

(Sheet 3 of 4)

Table 17 (Concluded)

Well No.	Date	Calcium	Magnesium	Manganese	Total Iron	Nitrate/Nitrite Nitrogen	Sulfate	Chloride
WES3 (Cont.)	09/05/97	122	47.7	<0.001	<0.20	2.88	3.93	<0.38
	10/01/97	95.2	52.4	<0.001	<0.20	4.68	3.18	<0.38
	10/30/97	103	53	<0.001	<0.20	2.65	90.3	6.71
	12/04/97	111	51.2	0.002	<0.20	1.93	71	18.5
	01/14/97	107	50.2	0.001	<0.20	1.73	92.4	6.38

(Sheet 4 of 4)

Correlations with explosives data. Contaminant concentration data were analyzed with concurrent geochemical parameter measurements (Ca, Fe, Mg, Mn, TOC, NO_2/NO_3, SO_4, and Cl) and water levels to determine whether contaminants were correlated with any of these parameters (Table 18). Both Pearson's *r* and Spearman's nonparametric correlation analyses were conducted, because of the large number of less than detection limit values for explosives. Correlations were considered significant only when both analyses produced significant results ($P < 0.05$). Most of the significant correlations occurred in MW173, and all of those

Table 18
Significant Correlations ($P < 0.05$ for both Pearson's r and Spearman's Rho) Between Geochemical Parameters and Contaminant Concentrations, Rounds 1-8 at JAAP

Geochemical Parameter	Significant Positive Correlations (r, P, n)		Significant Negative Correlations (r, P, n)	
	Contaminant(s)	Well	Contaminant(s)	Well
Ca	--	--	RDX (-0.758, 0.0292, 8)	MW131
			TNB (-0.902, 0.0022, 8)	MW173
Fe	--	--	--	--
Mg	DNA (0.769, 0.0256, 8)	MW172	RDX (-0.840, 0.0090, 8)	MW131
	DNA (0.893, 0.0413, 5)	WES1	TNB (-0.894, 0.0028, 8)	MW173
Mn	TNB (0.919, 0.0273, 5) TNT (0.998, 0.0001, 5)	WES1	--	--
TOC	2ADNT (0.902, 0.0022, 8)	MW131	HMX (-0.719, 0.0445, 8) TNT (-0.740, 0.0359, 8) 4ADNT (-0.913, 0.0015, 8) 2ADNT (-0.923, 0.0011, 8)	MW173
NO_2/NO_3	TNB (0.972, 0.0056, 5) TNT (0.918, 0.0277, 5)	WES1	--	--
SO_4	--	--	TNT (-0.834, 0.0100, 8)	MW173
Cl	RDX (0.722, 0.0430, 8)	MW172	4ADNT (-0.963, 0.0083, 5)	WES3
Water Level	RDX (0.835, 0.0100, 8) TNB (0.853, 0.0071, 8) TNT (0.789, 0.0200, 8)	MW172	2ADNT (-0.728, 0.0406, 5)	MW131
			TNB (-0.873, 0.0046, 8) TNT (-0.896, 0.0026, 8)	MW173

(correlations with Ca, Mg, TOC, SO_4, and water level) were negative. More explosives data correlated with TOC than with any other parameter. However, these results, taken as a whole, indicate no consistent influence of geochemical characteristics on explosives concentrations.

Results of Cone Penetrometry: JAAP

Results of cone penetrometer lithologies indicated a persistent clay layer of glacial origins underlying most of Site L1. Typically, the clay was first encountered at 1.5 to 2.5 ft below ground level and was 5 to 10 ft thick (Figures 7, 17, and 18). The hydraulic gradients varied from 0.03 for the glacial materials to 0.0007 for the bedrock. The water levels in the updip areas of the site were about 1 m higher than those near Prairie Creek, thereby suggesting a downward flow into the bedrock. Water levels from wells completed near Prairie Creek suggested little downward movement and indicated that groundwater flows horizontally along permeable zones until discharged into Prairie Creek. These observations are consistent with flow direction reported previously (Dames and Moore, Inc. 1993).

Analyses of soil samples collected at sites along the transects indicated that most of the contamination was concentrated on top of the clay layer and was restricted to the ridge and furrow system (Table 19). Only 11 of the 65 samples showed concentrations above detection limits. Ten of these were on Transects 1 and 2 in the ridge and furrow system. The other was on Transect 4. The highest concentration was for TNT (2.39 mg kg^{-1}). However, higher concentrations were detected in surface soil samples collected in the ridge and furrow system (see below).

Soil samples collected by CPT were primarily sandy loam with moderate to high amounts of organic carbon. Soil pH values tended to be neutral in the surface layers, but more alkaline with depth. Nutrients (nitrogen, sulfate, and phosphorus) were adequate to support microbial activity (Table 20).

Results of Surface Soil Analyses: JAAP

Concentrations of TNT in surface soil samples in the ridge and furrow system were moderate to low (Table 21). TNT levels approached or exceeded 1,000 mg kg^{-1} in only three samples, JS-8, JS-10, and JS-19, while the concentrations at all of the remaining locations averaged 37.09 ± 17.24 mg kg^{-1} (± standard error). RDX was found at low levels in five surface soil samples, while TNB was present in most of the surface soils. The TNT transformation products, 2,6DANT, 2,4DANT, 1,4-dinitrobenzene (1,4DNB), 1,3-dinitrobenzene (1,3DNB), 2ADNT, 4ADNT, 2,4DNT, and 2,6DNT, occurred sporadically in the μg kg^{-1} (ppb) to low mg kg^{-1} (ppm) level. TNT was found in all of the Prairie Creek stream sites; the only other analytes detected were RDX, TNB, and 2,6DANT at low concentrations and at only one site (Table 22).

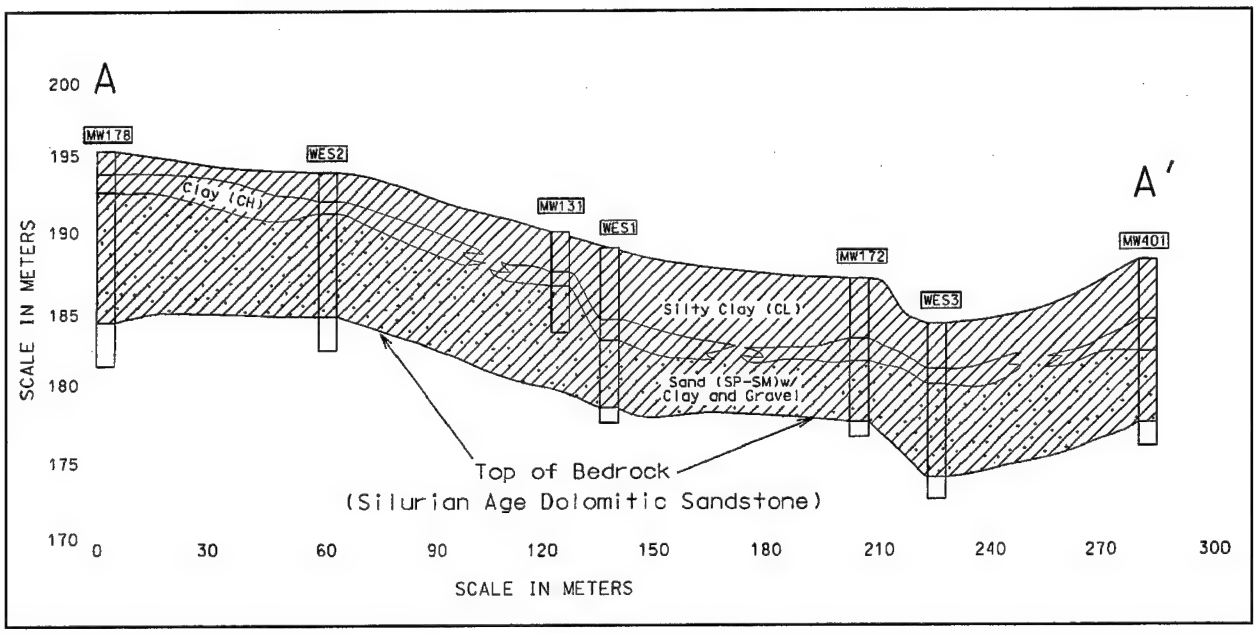

Figure 17. Geological Cross Section A-A′ at JAAP

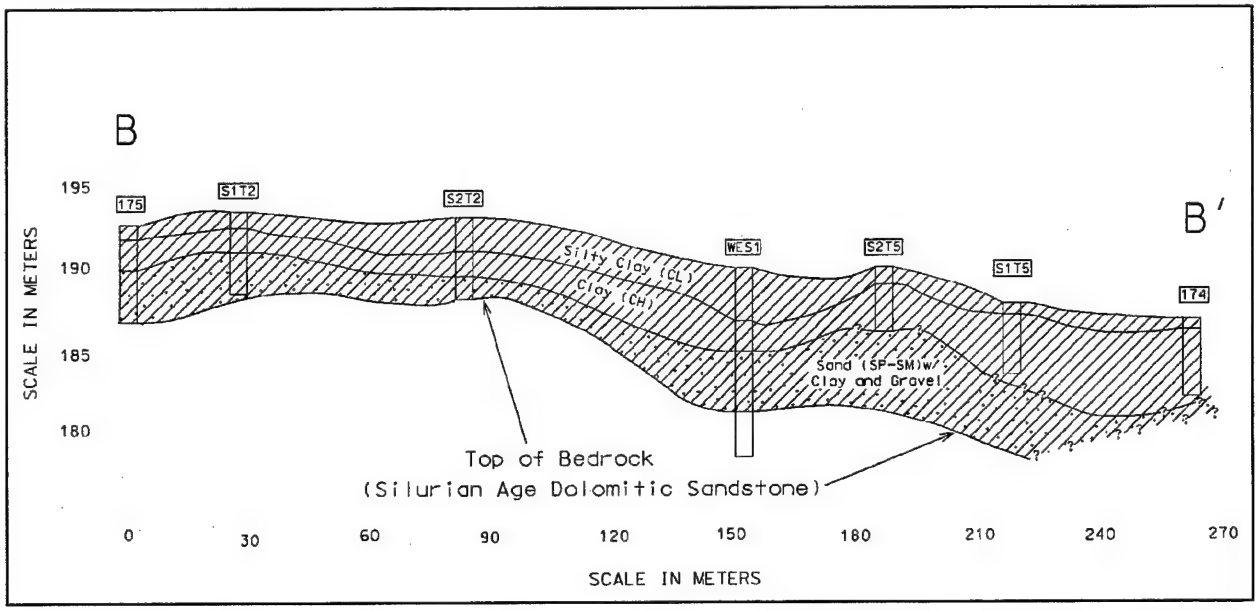

Figure 18. Geological Cross Section B-B′ at JAAP

Conclusions

Declining concentrations of explosives over the 2-year monitoring period were documented at LAAP. Results support the first line of evidence required under EPA guidance for verification of monitored natural attenuation, i.e., declining

Table 19
Concentration (μg kg^{-1}) of Explosives and Explosives Degradation Products in Soils Collected by CPT from Joliet Army Ammunition Plant[1]

Sample	RDX	TNB	TNT	4ADNT	2ADNT	2,4DNT	2,6DNT	2,6DANT	4,4AZ0XY	35DNA
S1-T1-2-3.1m	0.047J[2]	0.796	0.072J	0.208	0.353	0.028J	0.1DL	0.1DL	<1.00[3]	0.158
S1-T1-3-4.6m	0.1DL	0.167	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	<1.00	0.069
S1-T1-4-4.7m	0.1DL	0.148	0.042J	0.055J	0.073J	0.1DL	0.1DL	0.1DL	<1.00	0.074J
S1-T1-5-5.2m	0.1DL	0.019J	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	<1.00	0.014J
S3-T1-2-Surface	0.1DL	0.251	0.991	0.900	1.130	0.219	0.1DL	0.1DL	0.105J	0.126
S3-T1-3-1.7m	0.1DL	0.1DL	0.1DL	0.061J	0.071J	0.1DL	0.1DL	0.1DL	<1.000	0.1DL
S3-T1-4-4.1m	0.1DL	0.1DL	0.1DL	0.1DL	0.02J	0.1DL	0.1DL	0.1DL	<1.000	0.1DL
S3-T1-VP-0.2m[4]	0.1DL	0.1DL	0.580	0.269	0.504	0.1DL	0.130	0.158	ND[5]	ND
S4-T1-2-Surface	0.1DL	0.1DL	0.1DL	0.013J	0.1DL	0.1DL	0.1DL	0.1DL	<1.00	0.1DL
S2-T2-4-4.5m	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	<1.000	0.031J
S3-T2-VP-0.2[4]	0.1DL	1.520	0.041	0.1DL	0.115	0.1DL	0.1DL	0.1DL	ND	ND
S3-T2-VP-0.9m[4]	0.1DL	0.004	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	ND	ND
S3-T2-VP-1.5m[4]	0.1DL	0.032	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	ND	ND
S3-T2-VP-2.7m[4]	0.1DL	0.089	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	ND	ND
S3-T2-VP-3.4m[4]	0.1DL	0.251	0.1DL	0.1DL	0.043	0.1DL	0.1DL	0.1DL	ND	ND
S2-T4-2-Surface	0.1DL	0.1DL	0.1DL	0.043J	0.022J	0.1DL	0.1DL	0.1DL	<1.00	0.031J
S3-T4-3A & 3B-2.7m	0.1DL	0.1DL	0.1DL	0.017J	0.1DL	0.1DL	0.1DL	0.1DL	<1.00	0.1DL
S3-T4-4B & 4C-3.5m	0.464	0.964	2.39	0.442	0.484	0.1DL	0.1DL	0.1DL	<1.00	0.196

[1] Results presented are for samples in which at least one analyte was detected (including J values as detections); 18/65 samples.
[2] J indicates quantifiable values below statistically reliable detection limits.
[3] Detection limits.
[4] All vertical profile samples were analyzed by a different laboratory, but by the same methods. Analytes always below detection limits in vertical profile samples included 2,4DANT, 1,4DNB, 1,3DNB, nitrobenzene, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene.
[5] Not done.

contaminant mass. Methods were developed to optimize accuracy and minimize variability between sampling events, so that trends in concentration over time were readily demonstrated and reliable. None of the geochemical characteristics of the site correlated with explosives concentrations. Therefore, monitoring geochemical parameters provides no evidence of natural attenuation processes.

Sampling methods developed at LAAP were verified by application at JAAP. Although the sampling period at JAAP was limited to 9 months, about 20 percent of the wells exhibiting concentrations of explosives above detection limits showed significant declines. Several wells (about 11 percent) showed increases. These were limited to transformation products of TNT and TNB except in the last

Table 20
Particle Size and Geochemical Composition of CPT Soil Samples at Joliet Army Ammunition Plant

Sample	pH	Particle Size, %				Geochemical Parameters, mg kg[1]						
		Sand	Silt	Clay	Texture[1]	Total P[2]	TOC[3]	TKN[4]	NO_2-N[5]	NO_3-N[6]	NH_4-N[7]	Sulfate
S1-T1-2-3.0m	8.2	67.5	20	12.5	Sandy loam	314	8,880	15.2	2	4	ND[8]	11
S1-T1-3-4.6m	7.9	67.5	22.5	10	Sandy loam	281	10,600	<3.66[9]	2	3	ND	18
S1-T1-4-4.7m	7.8	67.5	20	12.5	Sandy loam	311	9,830	<4.51	1	1	ND	182
S1-T1-5-5.2m	7.8	60	32.5	7.5	Sandy loam	257	9,480	<9.43	1	2	ND	174
S2-T1-2-0.9m	8.1	60	22.5	17.5	Sandy loam	316	9,020	11	1	3	ND	13
S2-T1-3-4.9m	8.3	75	17.5	7.5	Loamy sand	323	9,430	19.6	1	3	ND	10
S2-T1-4-6.3m	7.8	42.5	52.5	5	Silt loam	243	11,600	<11.3	1	3	ND	214
S2-T1-5A-0m	7.2	47.5	32.5	20	Loam	370	12,100	29.2	1	3	ND	26
S3-T1-2-SURFACE	7.9	52.5	30	17.5	Loam	361	14,500	87.8	1	1	ND	8
S3-T1-3-1.7m	8	62.5	20	17.5	Sandy loam	330	7,500	13.5	1	3	ND	13
S3-T1-4-4.1m	8	85	10	5	Loamy sand	239	3,660	<10.6	1	1	ND	10
S3-T1-VP-0.2m[10]	7.8	57.5	27.5	15	Sandy loam	148	18,900	291	16.4	4.30	18.5	3.2
S3-T1-VP-1.0m[10]	7.4	47.5	35	17.5	Loam	161	9,460	181	17.0	4.45	1.36	41.1
S3-T1-VP-2.0m[10]	8.4	57.5	22.5	20	Sandy loam	266	32,600	225	9.58	4.40	6.80	42.5
S3-T1-VP-2.8m[10]	8.4	55.0	22.5	22.5	Sandy clay loam	240	33,200	248	6.49	<1.05	11.0	43.2

(Sheet 1 of 4)

[1] Texture classification according to Buckman and Brady (1969).
[2] Phosphorous.
[3] Total organic carbon.
[4] Total Kjeldahl nitrogen.
[5] Nitrite nitrogen.
[6] Nitrate nitrogen.
[7] Ammonia nitrogen.
[8] Not done.
[9] Less than detection limits.
[10] All vertical profile samples were from independent penetrations and were analyzed by a different laboratory using slightly different methods.
[11] Insufficient sample for analysis.

Table 20 (Continued)

Sample	pH	Particle Size, %				Geochemical Parameters, mg kg[1]						
		Sand	Silt	Clay	Texture[1]	Total P[2]	TOC[3]	TKN[4]	NO_2-N[5]	NO_3-N[6]	NH_4-N[7]	Sulfate
S3-T1-VP-4.3m[10]	8.7	80.0	15.0	5.0	Loamy sand	151	51,300	54.9	9.05	<0.471	3.00	65.2
S3-T1-VP-4.9m[10]	8.4	57.5	32.5	10.0	Sandy loam	182	42,300	160	8.19	1.40	<1.12	76.8
S4-T1-3-1.6m	8	52.5	30	17.5	Loam	331	9,260	15.8	1	4	ND	12
S4-T1-4-2.9m	7.9	55	25	20	Loam	314	8,540	<11.1	1	3	ND	17
S4-T1-5-5.4m	7.4	52.5	27.5	20	Loam	355	13,200	36.4	1	3	ND	247
S4-T1-6-0.6m	6.6	47.5	30	22.5	Loam	287	4,490	26.2	2	1	ND	13
S4-T1-VP-0.2m[10]	7.1	50.0	40.0	10.0	Sandy loam	257	34,600	618	18.1	3.69	16.3	32.3
S4-T1-VP-1.1m[10]	5.9	50.0	27.5	22.5	Sandy clay loam	169	5,740	103	7.98	5.68	3.56	39.5
S4-T1-VP-1.8m[10]	8.2	55.0	25.0	20.0	Sandy clay loam	267	27,400	240	6.40	4.34	3.63	101
S4-T1-VP-2.4m[10]	8.3	52.5	27.5	20.0	Sandy clay loam	299	30,800	332	8.55	4.24	7.97	61.1
S4-T1-VP-3.1m[10]	8.3	52.5	25.0	22.5	Sandy loam	281	34,800	267	7.75	4.48	9.41	50.4
S4-T1-VP-5.6m[10]	8.4	52.5	25.0	22.5	Sandy clay loam	355	35,100	433	11.3	4.78	7.18	169
S1-T2-2-1.6m	8.1	70	20	10	Sandy loam	270	6,560	15	1	4	ND	16
S1-T2-3A-3.4m	7.8	65	17.5	17.5	Sandy loam	312	11,500	<9.60	2	1	ND	513
S1-T2-4A-4.0m	8.1	65	22.5	12.5	Sandy loam	208	6,240	<11.9	2	3	ND	17
S1-T2-5A-4.5m	8	67.5	20	12.5	Sandy loam	257	8,160	39.3	1	1	ND	53
S2-T2-2-1.1m	8.1	65	17.5	17.5	Sandy loam	330	8,380	<11.5	1	1	ND	13
S2-T2-3-2.4m	8.2	65	17.5	17.5	Sandy loam	328	9,400	<11.0	1	4	ND	11
S2-T2-4-4.5m	8.1	72.5	20	7.5	Loamy sand	313	8,460	<11.7	1	3	ND	7
S3-T2-VP-0.2[10]	8.6	47.5	27.5	25.0	Sandy clay loam	332	36,900	334	5.35	5.35	9.25	115
S3-T2-VP-0.9[10]	8.5	45.0	27.5	27.5	Clay loam	342	32,300	327	13.9	13.9	12.3	549

Table 20 (Continued)

Sample	pH	Particle Size, %				Geochemical Parameters, mg kg[1]						
		Sand	Silt	Clay	Texture[1]	Total P[2]	TOC[3]	TKN[4]	NO_2-N[5]	NO_3-N[6]	NH_4-N[7]	Sulfate
S3-T2-VP-1.5[10]	8.6	47.5	27.5	25.0	Loam	347	34,300	317	7.85	7.85	7.69	281
S3-T2-VP-2.7[10]	8.5	50.0	22.5	27.5	Sandy loam	154	57,300	98.6	19.7	16.7	<1.12	155
S3-T2-VP-3.4[10]	8.4	85.0	10.0	5.0	Loamy sand	382	36,800	384	16.1	7.85	9.89	251
S1-T3-2-0.6m	6.3	45	37.5	17.5	Loam	396	5,960	63.1	2	3	ND	38
S1-T3-3-1.9m	8.1	57.5	25	17.5	Sandy loam	325	8,310	<11.9	2	4	ND	25
S1-T3-4-3m	8.1	72.5	20	7.5	Loamy sand	280	4,910	<10.1	1	3	ND	16
S2-T3-2-0.6m	7.9	52.5	30	17.5	Loam	326	9,300	12	1	2	ND	11
S2-T3-3-1.6m	8.3	52.5	22.5	25	Sandy clay loam	318	9,040	<10.2	1	4	ND	10
S2-T3-4-2.6m	8.2	72.5	15	12.5	Loamy sand	261	6,680	<10.8	1	1	ND	10
S1-T4-3A-1.5m	7.5	70	12.5	17.5	Sandy loam	306	6,100	27.4	2	4	ND	477
S1-T4-4B-2.2m	7.2	77.5	15	7.5	Loamy sand	259	8,130	31	1	IS[11]	ND	615
S1-T4-4C-2.2m	7.3	85	15	0	Loamy sand	504	6,050	41.3	1	3	ND	282
S2-T4-2-SURFACE	7.3	55	37.5	7.5	Sandy loam	527	25,700	158	1	5	ND	17
S2-T4-3&3A-1.0m	7.6	80	17.5	2.5	Loamy sand	327	12,500	43.4	1	3	ND	96
S2-T4-4A&4C-1.7m	8.1	85	12.5	2.5	Loamy sand	185	3,940	12.7	2	3	ND	44
S3-T4-2-0.2m	7.8	67.5	25	7.5	Sandy loam	217	9,890	23.6	1	4	ND	7
S3-T4-5A-0.6m	7.3	52.5	40	7.5	Sandy loam	559	11,200	90.6	1	1	ND	9
S3-T4-3A&3B-2.7m	8	77.5	15	7.5	Loamy sand	409	5,670	18.9	2	3	ND	7
S3-T4-4B8&4C-3.5m	8	77.5	17.5	5	Loamy sand	203	9,780	<11.5	1	2	ND	32
S1-T5-2-1.9m	8.3	60	20	20	Sandy clay loam	303	6,420	16.5	2	3	ND	10
S1-T5-3-2.6m	8.1	72.5	17.5	10	Sandy loam	251	6,990	<8.27	2	1	ND	58

(Sheet 3 of 4)

Table 20 (Concluded)

Sample	pH	Particle Size, %				Geochemical Parameters, mg kg[1]						
		Sand	Silt	Clay	Texture[1]	Total P[2]	TOC[3]	TKN[4]	NO_2-N[5]	NO_3-N[6]	NH_4-N[7]	Sulfate
S1-T5-4A-3.81m	7.7	45	35	20	Loam	294	15,100	55.6	2	1	ND	632
S2-T5-2-0.8m	8.1	62.5	20	17.5	Sandy loam	391	8,700	23.3	1	4	ND	10
S2-T5-3-2.3m	8.3	82.5	10	7.5	Loamy sand	232	3,690	24.3	2	4	ND	10
S2-T5-4-3.2m	8	72.5	22.5	5	Loamy sand	230	6,010	< 10.2	2	4	ND	5
S2-T5-5-SURFACE	7.1	50	32.5	17.5	Sandy loam	345	9,400	44.7	1	4	ND	5
S1-T6-2-1.8m	8.2	50	27.5	22.5	Loam	325	7,570	11.6	1	3	ND	10
S1-T6-3-3.8m	8.2	70	22.5	7.5	Loamy sand	295	5,390	19.7	1	2	ND	15
S1-T6-3B-3.1m	8.1	62.5	22.5	15	Sandy loam	264	8,820	12.3	1	4	ND	13
S1-T6-4-7.0m	8.1	87.5	10	2.5	Loamy sand	190	9,180	<10.3	1	4	ND	24
S2-T6-2-1.2m	6.3	57.5	30	12.5	Sandy loam	442	2,140	<12.4	1	3	ND	10
S2-T6-3-2.9m	8.0	75	17.5	7.5	Loamy sand	492	7,360	<10.9	1	4	ND	13
S2-T6-4-4.3m	7.6	80	15	5	Loamy sand	281	9,050	<10.5	1	3	ND	9
S1-T7-2-SURFACE	7.7	60	27.5	12.5	Sandy loam	370	9,340	76.3	1	5	ND	6
S1-T7-3-0.9m	8.1	62.5	15	22.5	Sandy clay loam	297	5,480	17.3	2	3	ND	15
S1-T7-4-2.2m	8.1	60	15	25	Sandy clay loam	334	6,910	<11.4	1	4	ND	10
S1-T7-5-5.6m	8.1	70	17.5	12.5	Sandy loam	227	9,230	23.8	1	2	ND	14

Table 21
Explosives Concentrations in Surface Soils Collected Across the Ridge and Furrow System (mg kg^{-1})

Sample	2,6ADNT	2,4ADNT	RDX	TNB	1,4DNB	1,3DNB	TNT	2ADNT	4ADNT	2,4DNT	2,6DNT	3NT	4NT
JS-1	0.1DL[1]	0.1DL	0.1DL	5.01	0.1DL	0.1DL	1.98	1.61	0.32	0.1DL	0.1DL	0.1DL	0.1DL
JS-2	0.55	0.1DL	0.1DL	370.79	0.55	0.1DL	13.66	3.58	0.1DL	0.23	0.1DL	0.1DL	0.1DL
JS-3	0.1DL	0.1DL	0.1DL	4.61	0.1DL	0.1DL	5.58	0.71	0.14	0.1DL	0.1DL	0.1DL	0.1DL
JS-4	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	7.25	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL
JS-5	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL
JS-6	0.1DL	0.1DL	0.1DL	1.03	0.1DL	0.1DL	2.97	2.14	2.65	0.1DL	0.1DL	0.1DL	0.1DL
JS-7	0.1DL	0.1DL	0.1DL	1.94	0.1DL	0.1DL	100.94	10.31	0.1DL	1.60	0.1DL	0.1DL	0.1DL
JS-8	0.1DL	0.1DL	0.16	70.62	2.69	3.66	10,056.00	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL
JS-9	0.1DL	0.1DL	0.1DL	0.99	0.1DL	0.1DL	3.72	3.83	0.24	0.1DL	0.1DL	0.1DL	0.1DL
JS-10	0.1DL	0.1DL	0.09	35.36	0.35	0.38	1,676.57	19.31	16.17	1.83	0.1DL	0.1DL	0.12
JS-11	0.1DL	0.1DL	0.1DL	1.05	0.1DL	0.1DL	2.87	3.77	0.59	0.57	0.1DL	0.1DL	0.1DL
JS-12	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.74	1.29	0.10	0.1DL	0.1DL	0.1DL	0.1DL
JS-13	0.1DL	0.1DL	0.1DL	1.15	0.1DL	0.1DL	2.37	1.72	0.1DL	1.42	0.1DL	0.1DL	0.1DL
JS-14	0.1DL	0.1DL	0.1DL	0.69	0.1DL	0.1DL	3.18	1.01	0.1DL	0.34	0.1DL	0.1DL	0.1DL
JS-15	0.19	0.1DL	0.01	1.06	0.1DL	0.1DL	2.48	0.46	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL
JS-16	0.1DL	0.1DL	0.1DL	0.23	0.1DL	0.1DL	2.27	0.62	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL
JS-17	0.1DL	0.1DL	0.1DL	0.47	0.1DL	0.1DL	4.71	1.96	0.56	0.1DL	0.1DL	0.1DL	0.1DL
JS-18	0.1DL	0.1DL	0.1DL	0.36	0.1DL	0.1DL	4.76	1.04	0.10	0.1DL	0.1DL	0.1DL	0.1DL
JS-19	0.1DL	0.1DL	0.1DL	7.48	0.1DL	0.46	966.63	22.25	26.0	4.14	1.62	0.1DL	0.92

(Continued)

[1] Below detection limit.

Table 21 (Concluded)

Sample	2,6ADNT	2,4ADNT	RDX	TNB	1,4DNB	1,3DNB	TNT	2ADNT	4ADNT	2,4DNT	2,6DNT	3NT	4NT
JS-20	0.1DL	0.1DL	0.1DL	1.60	0.1DL	0.1DL	16.61	11.16	3.57	1.66	0.1DL	0.1DL	0.1DL
JS-21	0.1DL	0.1DL	0.1DL	22.70	1.69	1.41	497.82	22.14	11.41	3.72	0.01	0.1DL	0.1DL
JS-22	0.1DL	0.1DL	0.1DL	11.97	0.17	0.27	287.90	13.59	6.87	2.43	0.1DL	0.1DL	0.1DL
JS-23	0.1DL	0.1DL	0.1DL	2.22	0.1DL	0.1DL	3.83	9.37	2.24	2.79	0.1DL	0.1DL	0.1DL
JS-24	0.13	0.1DL	0.1DL	0.16	0.1DL	0.1DL	0.61	0.23	0.1DL	0.1DL	0.1DL	0.10	0.1DL
JS-25	0.93	0.05	0.25	7.61	0.05	0.34	26.28	11.96	5.74	1.44	0.19	0.86	0.1DL
JS-26	0.12	0.1DL	0.1DL	3.41	0.1DL	0.1DL	3.50	3.77	1.31	0.10	0.1DL	0.32	0.1DL
JS-27	0.22	0.1DL	0.1DL	2.62	0.60	0.06	3.29	0.93	0.40	0.33	0.1DL	0.09	0.1DL
JS-28	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.37	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL	0.1DL
JS-29	0.11	0.1DL	0.50	14.40	0.17	0.54	283.39	14.61	7.28	3.13	0.73	1.14	0.1DL
JS-30	0.24	0.1DL	0.1DL	9.70	0.12	0.50	37.90	11.32	5.67	2.48	0.90	0.60	0.1DL

Table 22
Explosives Concentrations in Prairie Creek Samples (mg kg^{-1})[1]

Sample and Location	2,6ADNT	RDX	TNB	TNT
PC1	0.1DL[2]	0.1DL	0.1DL	2.55
PC2	0.1DL	0.1DL	0.1DL	0.80
PC3	0.1DL	0.1DL	0.1DL	1.58
PC4	0.1DL	0.1DL	0.1DL	0.66
PC5	0.1DL	0.1DL	0.1DL	1.89
PC6	0.59	0.35	0.01	6.06

[1] The contaminants 2ADNT, 4ADNT, 2,4DNT, 2,6DNT, 2,4DANT, 1,4DNB, and 1,3DNB were below detection limits. Therefore, they were omitted from the table.
[2] 0.1DL = Below detection limit.

sampling round where explosives concentrations increased in one well. Geochemical parameters were unrelated to explosives concentrations as observed at LAAP.

Definition of the contaminant plume was refined by cone penetrometry sampling at both sites. By coupling rapid laboratory "turn-around" with placement of the CPT, efficiency was optimized while minimizing analysis of uncontaminated samples beyond the plume. Lithology and contaminant data were used in the site conceptual and numerical models. The CPT also provided samples for development of biomarkers.

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Appendix
Chemical Analyses of Groundwater from the Louisiana Army Ammunition Plant

Appendix Table 1
Concentrations of Geochemical Parameters in Groundwater (mg L^{-1}) at LAAP

Well	Sampling Month[1]	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW012U	10	0.827	0.984	0.003	1.200	4.50	1.03	0.823	78.6	1.75	1.47
	13	1.6	0.611	0.006	0.375DL[3]	4.40	1.59	1.06	56.50	NS[4]	NS
	16	1.97	0.775	0.01	9.96	4.50	1.93	1.14	1.28	NS	NS
	19	2.86	0.218	0.007	0.375DL	4.40	3.16	1.44	8.95	NS	NS
	21	1.35	0.378	0.007	0.41	3.60	1.46	0.932	100.0	NS	NS
	24	1.37	0.613	0.009	0.57	3.70	1.46	0.942	125.0	2.30	1.63
MW014U	1	0.021	0.279	0.005DL	3.050	1.0DL	NS	NS	NS	NS	NS
	2	0.042	0.289	0.011	2.870	1.0DL	NS	NS	NS	NS	NS
	3	0.039	0.926	0.011	2.380	1.0DL	NS	NS	NS	NS	NS
	4	0.02DL	0.272	0.005	2.320	1.0DL	NS	NS	NS	NS	NS
	5	0.02DL	0.297	0.003	2.520	1.0DL	NS	NS	NS	NS	NS
	6	0.024	4.490	0.004	4.560	1.0DL	NS	NS	NS	NS	NS
	10	0.02DL	0.356	0.003	2.770	1.0DL	1.660	0.819	1.410	NS	NS
	13	0.02DL	1.52	0.003	4.44	1.0DL	1.84	0.754	3.09	NS	NS
	24	0.949	0.305	0.02800	2.38	1.0DL	3.19	1.56	1.57	0.05DL	0.244
MW032U	1	0.033	0.376	0.008	1.280	1.0DL	NS	NS	NS	NS	NS
	2	0.015DL	0.385	0.008	1.260	1.0DL	NS	NS	NS	NS	NS
	3	0.015DL	0.439	0.005DL	1.020	1.0DL	NS	NS	NS	NS	NS
	4	0.027	0.448	0.002DL	0.839	1.0DL	NS	NS	NS	NS	NS

(Sheet 1 of 17)

[1] Sampling was initiated February 1996 (Sampling Month 1).
[2] Total organic carbon.
[3] Detection limit.
[4] Not assayed.

Appendix Table 1 (Continued)

Well	Sampling Month[1]	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW032U (Cont.)	5	0.020DL	0.472	0.004	0.838	1.0DL	NS	NS	NS	NS	NS
	6	0.095	0.524	0.018	0.899	1.0DL	NS	NS	NS	NS	NS
	10	0.100DL	0.548	0.005DL	1.670	1.0DL	120.0	28.20	232.0	NS	NS
	13	0.020	0.974	0.002	0.81	1.0DL	127.0	28.20	303.0	NS	NS
	24	0.020	0.666	0.002	1.67	1.0DL	108.0	29.0	255.0	NS	NS
MW034U	1	0.085	0.020DL	0.117	19.63	3.00	NS	NS	NS	NS	NS
	2	0.072	0.020DL	0.112	19.90	3.00	NS	NS	NS	NS	NS
	3	0.029	0.020DL	0.104	19.20	4.70	NS	NS	NS	NS	NS
	4	0.149	0.092	0.091	21.20	4.90	NS	NS	NS	NS	NS
	5	0.020DL	0.079	0.094	19.80	2.90	NS	NS	NS	NS	NS
	6	0.020DL	0.488	0.107	15.80	5.40	NS	NS	NS	NS	NS
	10	0.10DL	0.020DL	0.073	18.20	2.20	442.0	216.0	1,800.0	NS	NS
	13	0.020DL	1.39	0.090	19.30	3.00	531.0	274.0	323.0	NS	NS
	24	0.023	0.064	0.132	17.70	1.40	456.0	232.0	1,993.0	NS	NS
MW036U	1	0.015DL	1.960	0.065	0.375DL	2.20	NS	NS	NS	NS	NS
	2	0.015DL	1.160	0.056	0.770	1.30	NS	NS	NS	NS	NS
	3	0.015DL	0.345	0.058	0.567	1.90	NS	NS	NS	NS	NS
	4	0.020DL	0.923	0.057	0.375DL	1.90	NS	NS	NS	NS	NS
	5	0.020DL	0.994	0.056	0.522	3.00	NS	NS	NS	NS	NS
	6	0.020DL	0.963	0.055	0.375DL	2.30	NS	NS	NS	NS	NS

(Sheet 2 of 17)

Appendix Table 1 (Continued)

Well	Sampling Month[1]	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW036U (Cont.)	10	0.100DL	1.520	0.038	1.300	1.80	1.00DL	0.404	5.670	NS	NS
	13	0.020DL	3.73	0.063	0.42	2.10	0.308	0.495	10.00	NS	NS
	24	0.020DL	0.384	0.080	0.79	1.70	0.523	0.449	1.57	NS	NS
MW037U	1	0.15DL	0.857	0.057	15.30	1.0DL	NS	NS	NS	NS	NS
	2	0.016	2.010	0.062	14.10	1.0DL	NS	NS	NS	NS	NS
	3	0.015DL	0.415	0.074	11.70	1.0DL	NS	NS	NS	NS	NS
	4	0.020DL	2.190	0.068	13.70	1.0DL	NS	NS	NS	NS	NS
	5	0.020DL	2.230	0.068	13.50	1.0DL	NS	NS	NS	NS	NS
	6	0.043	1.770	0.063	13.50	1.0DL	NS	NS	NS	NS	NS
	24	0.02DL	2.01	0.0610	16.30	1.0DL	3.34	3.63	6.7	NS	NS
MW038U	1	1.970	1.240	0.219	29.80	6.20	NS	NS	NS	NS	NS
	2	2.30	0.292	0.227	20.40	9.60	NS	NS	NS	NS	NS
	3	3.230	0.20DL	0.268	11.20	17.10	NS	NS	NS	NS	NS
	4	4.470	0.20DL	0.350	19.10	12.70	NS	NS	NS	NS	NS
	5	4.470	0.20DL	0.376	20.40	9.00	NS	NS	NS	NS	NS
	6	5.50	0.20	0.462	22.00	9.60	NS	NS	NS	NS	NS
	10	8.010	0.313	0.528	28.40	6.50	27.90	5.890	13.70	NS	NS
	13	5.91	2.81	0.384	36.80	4.80	17.30	4.16	16.20	NS	NS
	24	5.7	0.465	0.257	37.40	1.00DL	11.50	2.81	11.30	NS	NS

Apendix Table 1 (Continued)

Well	Sampling Month[1]	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW070U	1	0.016	0.074	0.005DL	2.380	1.00DL	NS	NS	NS	NS	NS
	2	0.015DL	0.089	0.005DL	2.360	1.00DL	NS	NS	NS	NS	NS
	3	0.015DL	0.755	0.005DL	2.090	1.00DL	NS	NS	NS	NS	NS
	4	0.026	0.213	0.002DL	1.970	1.00DL	NS	NS	NS	NS	NS
	5	0.020DL	0.226	0.002	1.840	1.00DL	NS	NS	NS	NS	NS
	6	0.020DL	1.290	0.001DL	1.850	1.00DL	NS	NS	NS	NS	NS
	10	0.020DL	0.586	0.005DL	2.360	1.00DL	91.60	39.70	307.0	NS	NS
	13	0.020DL	0.020DL	0.002	1.67	1.00DL	83.70	39.10	375.0	NS	NS
	24	0.020DL	0.204	0.002	2.42	1.00DL	107.00	51.20	420.0	NS	NS
MW083U	1	0.020	2.10	0.038	3.790	2.50	NS	NS	NS	NS	NS
	2	0.015DL	1.970	0.037	3.530	2.00	NS	NS	NS	NS	NS
	3	0.015DL	2.350	0.039	3.180	3.00	NS	NS	NS	NS	NS
	4	0.020DL	2.060	0.374	3.390	3.10	NS	NS	NS	NS	NS
	5	0.020DL	2.360	0.038	3.180	2.40	NS	NS	NS	NS	NS
	6	0.020DL	7.850	0.040	3.160	2.40	NS	NS	NS	NS	NS
	10	0.020DL	3.250	0.032	3.170	2.50	0.283	0.485	5.530	NS	NS
	13	0.020DL	1.64	0.027	2.46	1.60	0.305	0.310	5.640	NS	NS
	16	0.020DL	1.45	0.027	29.0	1.60	0.247	0.277	1.86	NS	NS
	19	0.020DL	2.39	0.024	4.39	1.70	2.00DL	1.00DL	19.70	NS	NS

Appendix Table 1 (Continued)

Well	Sampling Month[1]	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW083U (Cont.)	21	0.020DL	1.04	0.025	14.90	1.40	0.201	0.263	4.23	NS	NS
	24	0.020DL	1.04	0.022	2.88	1.10	0.227	0.253	3.87	NS	NS
MW085U	1	0.017	1.570	0.142	1.280	10.60	NS	NS	NS	NS	NS
	2	0.015DL	6.940	0.133	1.270	9.90	NS	NS	NS	NS	NS
	3	0.015DL	1.460	0.152	0.924	11.60	NS	NS	NS	NS	NS
	4	0.020DL	1.260	0.142	0.863	10.00	NS	NS	NS	NS	NS
	5	0.020DL	1.430	0.142	0.892	18.10	NS	NS	NS	NS	NS
	6	0.020DL	2.020	0.136	0.962	10.20	NS	NS	NS	NS	NS
	10	0.020DL	1.380	0.138	1.660	10.40	2.880	1.280	11.10	0.10DL	0.10DL
	13	0.020DL	1.32	0.126	0.88	8.80	2.47	1.01	5.38	NS	NS
	16	0.020DL	1.63	0.115	1.76	8.60	2.28	0.852	7.27	NS	NS
	19	0.020DL	1.31	0.117	0.375DL	7.50	2.48	1.00DL	0.375DL	NS	NS
	21	0.020DL	1.47	0.119	1.09	8.30	2.31	0.928	7.990	NS	NS
	24	0.020DL	1.89	0.112	0.95	7.20	2.09	0.864	6.71	0.05DL	0.05DL
MW097L	1	0.045	0.396	0.066	2.190	1.00DL	NS	NS	NS	NS	NS
	2	0.042	0.020DL	0.069	2.030	1.00DL	NS	NS	NS	NS	NS
	3	0.032	0.020DL	0.068	1.830	1.00DL	NS	NS	NS	NS	NS
	4	0.058	0.030	0.065	1.740	1.00DL	NS	NS	NS	NS	NS

(Sheet 5 of 17)

Appendix Table 1 (Continued)

Well	Sampling Month[1]	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW097L (Cont.)	5	0.046	0.020DL	0.065	1.60	1.00DL	NS	NS	NS	NS	NS
	6	0.038	0.047	0.062	1.88	1.00DL	NS	NS	NS	NS	NS
	10	0.048	0.020DL	0.057	2.48	1.00DL	48.80	29.80	71.90	NS	NS
	13	0.021	0.020DL	0.055	2.01	1.00DL	49.40	29.80	84.0	NS	NS
	16	0.037	0.020DL	0.055	76.1	1.00DL	53.1	30.5	2.97	NS	NS
	19	0.020DL	0.071	0.049	3.85	1.00DL	51.60	29.80	16.50	NS	NS
	21	0.028	0.020DL	0.049	0.70	1.00DL	52.70	31.10	74.90	NS	NS
	24	0.027	0.020DL	0.043	2.21	1.00DL	50.80	31.20	67.0	NS	NS
MW099U	1	0.015DL	1.870	0.012	2.050	1.00DL	NS	NS	NS	NS	NS
	2	0.015DL	0.020DL	0.017	2.090	1.00DL	NS	NS	NS	NS	NS
	3	0.015DL	0.367	0.009	1.820	1.00DL	NS	NS	NS	NS	NS
	4	0.037	0.180	0.004	1.530	1.00DL	NS	NS	NS	NS	NS
	5	0.020DL	0.195	0.007	1.570	1.00DL	NS	NS	NS	NS	NS
	6	0.020DL	0.151	0.002	1.840	1.00DL	NS	NS	NS	NS	NS
	10	0.020DL	0.105	0.004	2.250	1.00DL	180.0	74.70	510.0	NS	NS
	13	0.020DL	0.138	0.003	1.76	1.00DL	211.0	77.80	337.0	NS	NS
	16	0.020DL	0.020DL	0.007	3.35	1.00DL	167.0	80.1	790.0	NS	NS
	19	0.020DL	0.088	0.010	2.46	1.00DL	214.0	85.0	34.30	NS	NS
	21	0.020DL	0.111	0.009	18.60	1.00DL	207.0	87.30	621.0	NS	NS

Appendix Table 1 (Continued)

Well	Sampling Month[1]	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW099U (Conc.)	24	0.020DL	0.116	0.010	2.48	1.00DL	205.0	82.30	630.0	0.05DL	0.05DL
MW100L	1	0.015DL	6.860	0.071	7.030	1.90	NS	NS	NS	NS	NS
	2	0.015DL	8.990	0.075	6.850	2.00	NS	NS	NS	NS	NS
	3	0.015DL	7.160	0.073	6.960	2.00	NS	NS	NS	NS	NS
	4	0.020DL	6.00	0.065	8.450	2.00	NS	NS	NS	NS	NS
	5	0.020DL	6.840	0.059	7.670	2.00	NS	NS	NS	NS	NS
	6	0.020DL	0.681	0.066	8.280	2.00	NS	NS	NS	NS	NS
	10	0.020DL	11.20	0.062	7.310	2.10	28.80	8.390	31.90	NS	NS
	13	0.020DL	6.99	0.064	8.20	2.00	30.50	8.51	36.20	NS	NS
	16	0.02DL	4.16	0.061	8.04	1.9	28.2	8.27	37.5	NS	NS
	19	0.020DL	13.1	0.057	5.68	1.80	28.00	7.790	11.40	NS	NS
	21	0.020DL	4.44	0.059	0.56	1.70	29.70	8.58	29.60	NS	NS
	24	0.020DL	8.36	0.059	9.49	1.50	25.50	8.50	32.40	0.05DL	0.062
MW104U	1	0.015DL	3.540	0.292	5.670	17.10	NS	NS	NS	NS	NS
	2	0.015DL	3.440	0.261	5.690	17.10	NS	NS	NS	NS	NS
	3	0.015DL	2.50	0.312	5.290	18.40	NS	NS	NS	NS	NS
	4	0.020DL	2.070	0.286	5.710	18.40	NS	NS	NS	NS	NS
	5	0.020DL	2.460	0.294	6.180	24.60	NS	NS	NS	NS	NS
	6	0.020DL	4.330	0.299	5.920	18.20	NS	NS	NS	NS	NS

Appendix Table 1 (Continued)

Well	Sampling Month[1]	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW104U (Cont.)	10	0.020DL	2.760	0.288	5.780	17.90	6.750	4.150	58.50	0.346	0.411
	13	0.020DL	2.21	0.274	3.30	16.80	6.90	4.13	32.30	NS	NS
	16	0.020DL	2.55	0.264	7.83	17.50	6.51	3.67	59.9	NS	NS
	19	0.020DL	2.66	0.281	0.375DL	17.60	7.80	3.79	2.61	NS	NS
	21	0.020DL	2.6	0.266	6.39	16.60	6.62	3.94	59.0	NS	NS
	24	0.020DL	3.85	0.256	6.32	15.80	6.55	3.92	59.50	0.05DL	0.236
MW105L	1	0.015DL	2.880	0.069	2.930	2.880	NS	NS	NS	NS	NS
	2	0.015DL	3.220	0.055	2.410	2.10	NS	NS	NS	NS	NS
	3	0.015DL	2.820	0.062	2.290	2.40	NS	NS	NS	NS	NS
	4	0.020DL	2.830	0.053	1.60	2.20	NS	NS	NS	NS	NS
	5	0.020DL	8.730	0.053	1.70	2.00	NS	NS	NS	NS	NS
	6	0.020DL	0.178	0.054	1.710	2.00	NS	NS	NS	NS	NS
	10	0.020DL	3.650	0.044	2.470	2.20	42.30	12.30	52.10	NS	NS
	13	0.020DL	2.8	11.80	1.31	1.70	40.40	11.80	27.80	NS	NS
	16	0.020DL	2.67	0.046	1.89	2.0	42.7	11.3	51.8	NS	NS
	19	0.020DL	3.81	0.045	6.01	1.80	48.00	11.50	27.80	NS	NS
	21	0.020DL	2.48	0.048	2.48	1.90	51.0	13.40	52.80	NS	NS
	24	0.020DL	3.82	0.040	6.90	1.40	47.40	12.0	6.73	NS	NS
MW107L	1	0.015DL	0.653	0.005DL	0.730	1.00DL	NS	NS	NS	NS	NS

Appendix Table 1 (Continued)

Well	Sampling Month	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW107L (Cont.)	2	0.015DL	1.620	0.010	0.810	1.00DL	NS	NS	NS	NS	NS
	3	0.015DL	1.540	0.005DL	0.529	1.00DL	NS	NS	NS	NS	NS
	4	0.020DL	1.410	0.002DL	0.375DL	1.00DL	NS	NS	NS	NS	NS
	5	0.020DL	1.510	0.001DL	0.375DL	1.00DL	NS	NS	NS	NS	NS
	6	0.020DL	1.530	0.001DL	0.375DL	1.00DL	NS	NS	NS	NS	NS
	10	0.020DL	1.850	0.001DL	1.220	1.00DL	14.60	2.60	11.70	NS	NS
	13	0.020DL	1.51	0.001DL	0.44	1.00DL	24.20	3.42	11.20	NS	NS
	24	0.020DL	1.63	0.001DL	0.98	1.00DL	67.50	1.66	67.40	0.05DL	0.05DL
MW108L	10	0.020	3.100	0.264	5.030	2.00	32.1	13.6	63.5	0.255	0.366
	13	0.020DL	2.02	0.237	4.39	1.70	31.2	14.0	36.40	NS	NS
	16	0.028	1.83	0.233	4.93	1.50	31.0	12.80	68.3	NS	NS
	19	0.020DL	1.73	0.239	0.375	1.20	39.40	13.10	2.88	NS	NS
	21	0.020DL	1.68	0.218	3.91	1.20	32.10	13.40	65.40	NS	NS
	24	0.020DL	1.99	0.202	3.87	1.10	30.70	13.50	36.30	0.05DL	0.05DL
MW109U	1	0.024	0.549	0.152	1.260	4.50	NS	NS	NS	NS	NS
	2	0.105	3.720	0.189	1.880	4.40	NS	NS	NS	NS	NS
	3	0.020	2.790	0.195	1.810	5.10	NS	NS	NS	NS	NS
	4	0.020	3.250	0.186	1.890	4.90	NS	NS	NS	NS	NS
	5	0.020DL	3.390	0.184	1.970	10.50	NS	NS	NS	NS	NS

(Sheet 9 of 17)

Appendix Table 1 (Continued)

Well	Sampling Month	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW109U (Cont.)	6	0.020DL	11.70	0.190	1.870	5.00	NS	NS	NS	NS	NS
	10	0.030	5.990	0.196	2.340	4.90	1.290	2.040	54.30	0.078	0.10DL
	13	0.029	3.68	0.251	1.13	4.60	1.56	2.43	34.30	NS	NS
	16	0.020DL	2.96	0.255	2.11	3.00	1.11	2.77	32.1	NS	NS
	19	0.020DL	3.09	0.254	0.375	2.70	2.0DL	2.43	3.19	NS	NS
	21	0.020DL	3.24	0.267	0.84	3.40	0.924	2.39	61.40	NS	NS
	24	0.020DL	3.91	0.193	0.86	3.10	0.697	1.98	57.20	0.05DL	0.05DL
MW110L	1	0.339	5.590	0.655	1.580	2.50	NS	NS	NS	NS	NS
	2	0.257	8.480	0.516	1.260	2.40	NS	NS	NS	NS	NS
	3	0.172	6.270	0.481	1.420	2.90	NS	NS	NS	NS	NS
	4	0.178	4.90	0.484	1.530	2.80	NS	NS	NS	NS	NS
	5	0.158	4.610	0.459	1.390	2.90	NS	NS	NS	NS	NS
	6	0.192	8.2	0.504	1.530	2.90	NS	NS	NS	NS	NS
	10	0.172	8.680	0.432	2.120	3.0	17.40	6.650	43.20	0.088	0.348
	13	0.189	4.43	0.445	1.18	2.80	17.90	6.88	29.30	NS	NS
	16	0.154	3.78	0.398	44.8	2.70	17.20	6.12	2.36	NS	NS
	19	0.206	5.28	0.44	0.375DL	2.40	19.40	6.68	1.93	NS	NS
	21	0.184	4.1	0.446	1.82	2.30	18.70	6.88	45.70	NS	NS
	24	0.221	6.36	0.466	1.86	2.40	18.40	6.91	54.70	0.05DL	0.055

Appendix Table 1 (Continued)

Well	Sampling Month	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW111U	1	0.015DL	1.30	0.005DL	0.400	1.00DL	NS	NS	NS	NS	NS
	2	0.015DL	0.621	0.005DL	0.920	1.00DL	NS	NS	NS	NS	NS
	3	0.015DL	0.538	0.005DL	0.584	1.00DL	NS	NS	NS	NS	NS
	4	0.020DL	0.613	0.002DL	0.375DL	1.00DL	NS	NS	NS	NS	NS
	5	0.020DL	0.630	0.001DL	0.375DL	1.00DL	NS	NS	NS	NS	NS
	6	0.020DL	0.682	0.001DL	0.375DL	1.30	NS	NS	NS	NS	NS
	10	0.020DL	0.878	0.001DL	1.210	1.00DL	2.280	0.660	2.830	NS	NS
	13	0.020DL	0.09	0.001DL	5.11	1.00DL	25.30	3.42	2.430	NS	NS
	16	0.020DL	0.544	0.001DL	19.7	1.00DL	13.6	1.80	1.64	NS	NS
	19	0.020DL	1.12	0.001DL	3.87	1.00DL	7.21	1.00DL	16.40	NS	NS
	21	0.020DL	0.61	0.001DL	0.31	1.00DL	5.17	0.845	3.30	NS	NS
	24	0.02	0.020DL	0.001DL	12.30	1.00DL	30.20	3.43	1.58	NS	NS
MW112L	1	2.240	0.564	0.401	11.90	1.00DL	NS	NS	NS	NS	NS
	2	2.630	0.731	0.376	14.10	1.00DL	NS	NS	NS	NS	NS
	3	2.680	0.020DL	0.364	13.0	1.00DL	NS	NS	NS	NS	NS
	4	2.70	0.864	0.358	14.20	1.00DL	NS	NS	NS	NS	NS
	5	2.670	2.930	0.364	12.40	1.00DL	NS	NS	NS	NS	NS
	6	2.710	2.970	0.351	13.70	1.00DL	NS	NS	NS	NS	NS
	10	2.750	0.950	0.327	11.40	1.00DL	17.90	6.980	21.60	NS	NS

Appendix Table 1 (Continued)

Well	Sampling Month	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW112L (Cont.)	13	2.79	0.707	0.313	14.60	1.00DL	18.50	7.33	24.40	NS	NS
	16	0.020DL	1.16	0.354	12.0	1.00DL	18.7	6.87	23.3	NS	NS
	19	3.17	1.13	0.340	0.94	1.00DL	19.70	6.51	28.10	NS	NS
	21	3.09	0.966	0.340	12.80	1.00DL	19.60	7.43	23.60	NS	NS
	24	3.05	0.865	0.301	6.35	1.00DL	18.90	7.59	20.50	NS	NS
MW136L	1	0.015DL	0.020DL	0.122	2.560	1.00DL	NS	NS	NS	NS	NS
	2	0.015DL	0.020DL	0.130	2.72	1.00DL	NS	NS	NS	NS	NS
	3	0.015DL	0.020DL	0.144	2.410	1.00DL	NS	NS	NS	NS	NS
	4	0.032	0.029	0.146	2.460	1.00DL	NS	NS	NS	NS	NS
	5	0.028	0.020DL	0.154	2.440	1.00DL	NS	NS	NS	NS	NS
	6	0.020DL	0.027	0.155	2.540	1.00DL	NS	NS	NS	NS	NS
	24	0.023	0.020DL	0.138	2.84	1.00DL	45.7	20.5	24.7	NS	NS
MW137L	1	0.251	0.598	0.377	1.470	1.00DL	NS	NS	NS	NS	NS
	2	0.089	0.601	0.342	1.30	1.00DL	NS	NS	NS	NS	NS
	3	0.056	0.635	0.274	1.120	1.00DL	NS	NS	NS	NS	NS
	4	0.052	0.578	0.218DL	0.935	1.00DL	NS	NS	NS	NS	NS
	5	0.028	0.609	0.171	0.984	1.00DL	NS	NS	NS	NS	NS
	6	0.020DL	1.20	0.127	1.13	1.00DL	NS	NS	NS	NS	NS
	13	0.02	1.37	0.0990	1.01	1.00DL	68.80	25.30	247.0	NS	NS

Appendix Table 1 (Continued)

Well	Sampling Month	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW137L (Cont.)	24	0.09	0.683	0.214	1.43	1.00DL	80.30	26.30	201.0	NS	NS
MW138L	1	0.026	0.767	0.026	4.130	1.00DL	NS	NS	NS	NS	NS
	2	0.016	0.020DL	0.022	4.270	1.00DL	NS	NS	NS	NS	NS
	3	0.023	0.492	0.018	3.920	1.00DL	NS	NS	NS	NS	NS
	4	0.059	0.495	0.022	3.960	1.00DL	NS	NS	NS	NS	NS
	5	0.02DL	0.509	0.017	4.400	1.00DL	NS	NS	NS	NS	NS
	6	0.02DL	0.500	0.020	6.670	1.00DL	NS	NS	NS	NS	NS
	10	0.01DL	0.467	0.007	4.690	1.00DL	184.0	59.30	474.0	NS	NS
	13	0.02DL	0.020DL	0.021	4.77	1.00DL	160.0	58.20	588.0	NS	NS
	24	0.02DL	0.559	0.010	8.16	1.00DL	168.0	58.20	497.0	NS	NS
MW140U	1	0.084	0.277	0.245	1.020	3.30	NS	NS	NS	NS	NS
	2	0.025	0.239	0.233	1.29	3.40	NS	NS	NS	NS	NS
	3	0.027	5.560	0.242	1.15	3.70	NS	NS	NS	NS	NS
	4	0.059	0.220	0.233	4.090	3.70	NS	NS	NS	NS	NS
	5	0.057	0.215	0.260	1.160	5.20	NS	NS	NS	NS	NS
	6	0.020DL	8.370	0.210	0.991	3.80	NS	NS	NS	NS	NS
	10	0.041	0.214	0.185	2.00	3.70	34.60	41.30	280.0	0.124	0.352
	13	0.049	0.144	0.225	0.92	3.20	43.10	16.10	NS	NS	NS
	16	0.09	0.233	0.193	21.4	3.00	35.90	12.60	0.375DL	NS	NS

Appendix Table 1 (Continued)

Well	Sampling Month	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW140U (Cont.)	19	0.102	0.143	0.201	0.375DL	3.00	42.0	13.40	15.0	NS	NS
	21	0.097	0.228	0.234	1.19	2.90	45.40	16.40	340.0	NS	NS
	24	0.048	0.188	0.170	1.23	2.40	32.40	12.30	250.0	0.070	0.156
MW141L	1	0.024	7.580	0.212	11.80	4.30	NS	NS	NS	NS	NS
	2	0.015DL	5.680	0.218	9.410	5.30	NS	NS	NS	NS	NS
	3	0.015DL	7.690	0.209	10.40	5.70	NS	NS	NS	NS	NS
	4	0.020DL	6.630	0.217	12.0	6.0	NS	NS	NS	NS	NS
	5	0.020DL	5.390	0.229	12.40	6.0	NS	NS	NS	NS	NS
	6	0.022	7.77	0.224	13.50	6.0	NS	NS	NS	NS	NS
	10	0.025	14.60	0.209	9.410	5.40	23.0	8.240	38.50	0.126	0.381
	13	0.020DL	7.61	0.211	5.14	5.20	23.20	8.44	21.60	NS	NS
	16	0.02ODL	4.04	0.175	16.6	5.60	22.20	7.48	39.1	NS	NS
	19	0.020DL	13.8	0.196	0.375DL	5.50	24.80	38.70	1.73	NS	NS
	21	0.020DL	4.61	0.196	9.73	4.90	23.60	8.18	41.50	NS	NS
	24	0.020DL	9.88	0.192	8.65	5.0	23.60	8.39	37.50	0.05DL	0.055
MW142U	1	0.387	0.345	0.011	0.760	1.0DL	NS	NS	NS	NS	NS
	2	0.015DL	0.604	0.005DL	1.010	1.0DL	NS	NS	NS	NS	NS
	3	0.200	0.376	0.005DL	0.836	1.0DL	NS	NS	NS	NS	NS
	4	0.020DL	0.613	0.002DL	0.610	1.0DL	NS	NS	NS	NS	NS

Appendix Table 1 (Continued)

Well	Sampling Month[1]	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW142U (Cont.)	5	0.028	0.658	0.001DL	0.581	1.0DL	NS	NS	NS	NS	NS
	6	0.066	0.652	0.001DL	0.690	1.0DL	NS	NS	NS	NS	NS
	10	0.040	0.678	0.001DL	1.50	1.0DL	0.20DL	0.287	5.930	NS	NS
	13	0.109	0.675	0.001DL	0.68	1.0DL	0.836	0.308	8.83	NS	NS
	24	0.151	0.776	0.001DL	0.85	1.0DL	0.20DL	0.20DL	5.01	NS	NS
MW145L	1	0.060	1.690	0.048	3.610	1.0DL	NS	NS	NS	NS	NS
	2	0.063	0.020DL	0.051	3.640	1.0DL	NS	NS	NS	NS	NS
	3	0.047	0.020DL	0.069	3.260	1.0DL	NS	NS	NS	NS	NS
	4	0.088	0.020DL	0.069	3.740	1.0DL	NS	NS	NS	NS	NS
	5	0.086	0.020DL	0.059	3.390	1.0DL	NS	NS	NS	NS	NS
	6	0.057	0.021	0.049	3.510	1.0DL	NS	NS	NS	NS	NS
	10	0.10DL	0.020DL	0.044	3.890	1.0DL	57.10	32.0	30.20	NS	NS
	13	0.045	0.020DL	0.086	3.53	1.0DL	55.70	29.50	35.40	NS	NS
	24	0.02DL	2.09	0.004	4.06	2.80	33.80	8.56	32.40	NS	NS
MW146L	1	0.015DL	1.660	0.005DL	4.20	1.0DL	NS	NS	NS	NS	NS
	2	0.015DL	1.90	0.005DL	3.960	1.0DL	NS	NS	NS	NS	NS
	3	0.015DL	1.910	0.005DL	3.540	1.0DL	NS	NS	NS	NS	NS
	4	0.020DL	1.650	0.002	4.0	1.0DL	NS	NS	NS	NS	NS
	5	0.020DL	1.70	0.003	3.560	1.0DL	NS	NS	NS	NS	NS

(Sheet 15 of 17)

Appendix Table 1 (Continued)

Well	Sampling Month[1]	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW146L (Cont.)	6	0.020DL	1.540	0.002	3.760	1.0DL	NS	NS	NS	NS	NS
	10	0.10DL	1.090	0.005DL	3.730	1.0DL	22.90	5.440	16.40	NS	NS
	13	0.020DL	0.734	0.002	3.88	1.0DL	23.60	6.52	21.90	NS	NS
	24	0.105	0.020DL	0.055	4.50	1.0DL	57.0	30.60	28.40	NS	NS
MW168L	1	0.021	7.70	0.083	12.95	5.00	NS	NS	NS	NS	NS
	2	0.015DL	10.50	0.068	12.60	4.90	NS	NS	NS	NS	NS
	3	0.015DL	9.70	0.070	12.40	5.20	NS	NS	NS	NS	NS
	4	0.020DL	7.130	0.069	14.10	1.00DL	NS	NS	NS	NS	NS
	5	0.020DL	8.730	0.068	14.30	5.00	NS	NS	NS	NS	NS
	6	0.020DL	10.10	0.060	13.50	5.60	NS	NS	NS	NS	NS
	10	0.020DL	17.80	0.067	11.20	5.50	30.70	11.40	46.70	NS	NS
	13	0.020DL	4.51	0.068	13.30	5.50	30.30	11.60	27.80	NS	NS
	16	0.020DL	4.5	0.080	28.7	5.50	33.0	11.30	31.1	NS	NS
	19	0.020DL	14.1	0.069	1.46	5.60	34.70	11.20	1.89	NS	NS
	21	0.020DL	4.81	0.078	13.10	5.10	32.30	11.80	54.10	NS	NS
	24	0.020DL	11.3	0.085	12.70	5.00	32.0	12.0	37.40	NS	NS
MW171U	1	0.015	0.377	0.006	0.780	1.0DL	NS	NS	NS	NS	NS
	2	0.015DL	0.256	0.005	1.050	1.0DL	NS	NS	NS	NS	NS
	3	0.017	0.166	0.005	0.919	1.0DL	NS	NS	NS	NS	NS

Appendix Table 1 (Concluded)

Well	Sampling Month[1]	Iron	Nitrate/ Nitrite	Manganese	Sulfate	TOC[2]	Calcium	Magnesium	Chloride	Iron II	Iron III
MW171U (Cont.)	4	0.020DL	0.319	0.002DL	0.744	1.0DL	NS	NS	NS	NS	NS
	5	0.020DL	0.367	0.001DL	0.719	1.0DL	NS	NS	NS	NS	NS
	6	0.020DL	0.343	0.001DL	4.530	1.0DL	NS	NS	NS	NS	NS
	10	0.020DL	0.509	0.001DL	1.580	1.0DL	37.0	11.60	40.80	NS	NS
	13	0.020DL	0.358	0.001DL	0.81	1.0DL	40.70	12.0	52.90	NS	NS
	24	0.144	0.426	0.022	1.13	1.0DL	39.20	12.90	47.90	NS	NS
MW189U	1	0.035	2.320	0.013	3.130	1.00DL	NS	NS	NS	NS	NS
	2	0.020	0.124	0.018	2.240	1.00DL	NS	NS	NS	NS	NS
	3	0.015DL	0.020	0.009	2.710	1.00DL	NS	NS	NS	NS	NS
	4	0.020DL	0.140	0.007	2.980	1.00DL	NS	NS	NS	NS	NS
	5	0.020DL	0.128	0.008	2.840	1.00DL	NS	NS	NS	NS	NS
	6	0.020DL	0.138	0.008	2.670	1.00DL	NS	NS	NS	NS	NS
	10	0.028	0.397	0.009	2.260	1.00DL	4.470	0.995	6.750	NS	NS
	13	0.020DL	0.064	0.004	2.15	1.00DL	2.83	0.744	3.08	NS	NS
	24	0.027	0.128	0.005	1.84	1.00DL	4.70	0.848	4.80	NS	NS

(Sheet 17 of 17)

Appendix Table 2
Groundwater Concentrations ($\mu g\ L^{-1}$) of Explosives and Their Transformation Products in 15 Wells at LAAP

Well	Sampling Month[1]	TNT	RDX	HMX	2,4DNT	2,6DNT	4ADNT	2ADNT	2,4DANT	TNB	DNB	NB	3,5DNA	MNX	TNX	MNHMX
MW012U	10	2,610.0	2,180.0	235.0	90.80	0.20DL[2]	0.200DL	0.810	6.610	960.0	25.20	0.20DL	3.310	0.20DL	0.20DL	NS[3]
	13	2,540.0	2,640.0	227.0	62.00	0.20DL	0.200DL	7.63	2.00DL	895.0	18.20	0.20DL	5.94	0.20DL	0.20DL	NS
	16	2,460.0	2,590.0	200.0	77.00	0.20DL	0.200DL	8.02	2.00DL	1,140.0	19.20	0.20DL	0.20DL	0.20DL	0.20DL	NS
	19	989.0	1,240.0	100.0	29.60	0.20DL	6.43	5.73	2.00DL	505.0	6.69	0.20DL	5.60	0.20DL	0.20DL	NS
	21	1,889.0	1,860.0	172.0	51.80	0.20DL	0.200DL	5.13	2.00DL	895.0	12.50	0.20DL	6.11	0.20DL	0.20DL	NS
	24	2,110.0	2,300.0	175.0	65.0	0.20DL	0.200DL	4.50	2.98	1,340.0	15.40	0.20DL	4.30	0.14J[4]	2.03	5
MW014U	1	0.20DL	24.1	6.39	0.20DL	0.20DL	0.20DL	0.220	2.0DL	0.20DL	0.20DL	0.20DL	0.20DL	NS	NS	NS
	2	0.210	22.0	6.09	0.20DL	0.20DL	0.20DL	0.20DL	2.0DL	0.20DL	0.20DL	0.20DL	0.20DL	NS	NS	NS
	3	0.20DL	18.3	0.20DL	0.20DL	0.20DL	0.20DL	0.20DL	2.0DL	0.20DL	0.20DL	0.20DL	0.20DL	NS	NS	NS
	4	0.20DL	24.1	0.20DL	0.20DL	0.20DL	0.20DL	0.20DL	2.0DL	1.940	0.20DL	0.20DL	0.20DL	NS	NS	NS
	5	0.20DL	22.3	5.87	0.20DL	0.20DL	0.20DL	0.150J	2.0DL	0.20DL	0.20DL	0.20DL	0.20DL	NS	NS	NS
	6	0.20DL	22.8	6.33	0.20DL	0.20DL	0.20DL	0.20DL	2.0DL	0.20DL	0.20DL	0.20DL	0.20DL	NS	NS	NS
	10	0.20DL	20.9	5.50	0.20DL	0.20DL	0.20DL	0.20DL	2.0DL	0.20DL	0.20DL	0.20DL	0.20DL	NS	NS	NS
	13	0.20DL	16.7	4.31	0.20DL	0.20DL	0.20DL	0.20DL	2.0DL	0.20DL	0.20DL	0.20DL	0.20DL	0.20DL	0.20DL	NS
	24	0.20DL	18.9	4.58	0.20DL	0.20DL	0.20DL	0.20DL	2.0DL	0.20DL	0.20DL	0.20DL	0.20DL	0.20DL	0.20DL	2.50DL
MW083U	1	2,890.0	2,060.0	216.0	61.60	0.20DL	43.80	146.0	2.00DL	742.0	3.810	0.20DL	50.50	NS	NS	NS
	2	2,530.0	1,720.0	197.0	13.80	78.70	32.30	107.0	2.00DL	661.0	2.460	0.20DL	36.60	NS	NS	NS
	3	2,620.0	1,780.0	170.0	14.80	0.20DL	37.60	126.0	11.40	599.0	2.590	0.20DL	34.70	NS	NS	NS

(Sheet 1 of 8)

[1] Sampling was initiated February 1996 (Sampling Month 1).
[2] Detection limit.
[3] Not assayed.
[4] J values are below the statistically reliable detection limit.

Appendix Table 2 (Continued)

Well	Sampling Month[1]	TNT	RDX	HMX	2,4DNT	2,6DNT	4ADNT	2ADNT	2,4DANT	TNB	DNB	NB	3,5DNA	MNX	TNX	MNHMX
MW083U (Cont.)	4	2,520.0	1,620.0	175.0	52.50	0.20DL	41.30	150.0	2.00DL	624.0	2.630	0.20DL	39.40	NS	NS	NS
	5	2,840.0	1,950.0	190.0	58.60	0.20DL	46.70	164.0	2.00DL	678.0	2.470	0.20DL	46.20	NS	NS	NS
	6	2,330.0	1,680.0	212.0	43.0	0.20DL	62.50	182.0	3.050	701.0	2.640	0.20DL	46.70	NS	NS	NS
	10	2,190.0	1,400.0	152.0	13.30	0.20DL	37.0	138.0	2.00DL	580.0	1.750	0.20DL	35.20	NS	NS	NS
	13	1,780.0	840.0	102.0	0.50DL	0.20DL	26.10	111.0	2.00DL	418.0	0.83	0.20DL	21.40	0.20DL	0.20DL	NS
	16	2,000.0	757.0	105.0	9.83	0.20DL	23.50	105.0	2.00DL	514.0	1.61	0.20DL	24.0	0.20DL	0.20DL	NS
	19	2,180.0	938.0	142.0	75.90	0.20DL	19.90	115.0	2.00DL	602.0	1.36	0.20DL	25.0	0.20DL	0.20DL	NS
	21	1,990.0	573.0	121.0	71.10	0.20DL	21.0	93.0	2.00DL	574.0	1.55	0.20DL	21.10	0.20DL	0.20DL	NS
	24	1,710.0	344.0	89.50	11.90	0.20DL	18.60	82.20	0.84	483.0	1.46	6.55	20.60	1.61	3.13	2.76
MW085U	1	7,500.0	11,800.0	1,300.0	101.0	0.20DL	50.0	291.0	2.00DL	9,150.0	42.80	0.20DL	281.0	NS	NS	NS
	2	7,850.0	11,000.0	1,640.0	73.80	30.60	19.90	252.0	2.00DL	9,300.0	40.0	0.20DL	198.0	NS	NS	NS
	3	9,190.0	13,000.0	1,770.0	90.20	0.20DL	0.200DL	266.00	2.00DL	10,100.0	45.70	0.20DL	194.0	NS	NS	NS
	4	7,470.0	10,500.0	1,630.0	91.60	0.20DL	0.200DL	276.00	2.00DL	9,100.0	38.60	0.20DL	290.0	NS	NS	NS
	5	8,070.0	9,050.0	1,820.0	90.80	0.20DL	0.200DL	299.00	2.00DL	10,600.0	38.00	0.20DL	306.0	NS	NS	NS
	6	7,200.0	8,630.0	1,700.0	75.70	0.20DL	0.200DL	344.00	2.00DL	9,990.0	35.90	0.20DL	242.0	NS	NS	NS
	10	7,350.0	8,520.0	1,450.0	90.70	0.20DL	0.200DL	275.00	6.960	9,710.0	39.00	0.20DL	187.0	NS	NS	NS
	13	6,370.0	7,390.0	1,400.0	54.60	0.20DL	0.200DL	294.00	2.00DL	8,820.0	26.00	0.20DL	136.0	0.20DL	0.20DL	NS
	16	5,790.0	6,690.0	1,360.0	57.30	0.20DL	0.200DL	253.00	2.00DL	8,980.0	24.70	0.20DL	155.0	0.20DL	0.20DL	NS
	19	4,860.0	7,310.0	943.0	53.10	0.20DL	0.200DL	228.00	2.00DL	7,540.0	23.30	0.20DL	142.0	0.20DL	0.20DL	NS
	21	6,149.0	8,283.0	1,343.0	58.50	0.20DL	0.200DL	0.20DL	2.00DL	9,124.0	25.40	0.20DL	144.0	0.20DL	0.20DL	NS

Appendix Table 2 (Continued)

Well	Sampling Month[1]	TNT	RDX	HMX	2,4DNT	2,6DNT	4ADNT	2ADNT	2,4DANT	TNB	DNB	NB	3,5DNA	MNX	TNX	MNHMX
MW085U (Cont.)	24	5,320.0	6,810.0	1,120.0	58.30	0.20DL	0.200DL	214.00	3.64	8,330.0	25.80	0.20DL	151.0	9.48	0.20DL	37.5
MW100L	1	49.60	16.10	0.20DL	173.00	0.20DL	0.200DL	1.490	2.00DL	32.90	20.50	0.20DL	22.80	NS	NS	NS
	2	64.70	14.80	0.20DL	157.00	0.20DL	0.200DL	1.410	2.00DL	32.90	19.20	0.20DL	18.30	NS	NS	NS
	3	62.80	16.50	0.20DL	177.00	0.20DL	0.200DL	1.030	2.00DL	15.60	21.10	0.20DL	16.40	NS	NS	NS
	4	67.80	14.90	0.20DL	188.0	0.20DL	0.200DL	1.360	2.00DL	24.60	21.40	0.20DL	0.20DL	NS	NS	NS
	5	70.0	17.50	0.20DL	188.00	0.20DL	0.200DL	0.500	2.00DL	16.40	21.20	0.20DL	0.20DL	NS	NS	NS
	6	88.80	18.80	0.20DL	203.00	0.20DL	0.200DL	1.270	2.00DL	15.50	22.10	0.20DL	0.20DL	NS	NS	NS
	10	99.20	19.70	0.20DL	213.00	0.20DL	0.200DL	1.160	19.80	13.60	22.0	0.20DL	1.770	NS	NS	NS
	13	99.80	21.10	0.20DL	179.00	0.20DL	0.200DL	0.20DL	2.00DL	12.20	19.20	0.20DL	11.60	0.20DL	0.20DL	NS
	16	148.0	21.90	0.20DL	199.00	0.20DL	0.76	1.72	2.00DL	11.40	19.70	0.20DL	10.40	0.20DL	0.20DL	NS
	19	173.0	29.40	0.40	210.00	0.20DL	0.200DL	0.44	2.00DL	13.20	20.50	0.20DL	10.90	0.2ODL	0.2ODL	NS
	21	158.0	25.40	0.20DL	201.00	0.20DL	0.200DL	0.20DL	2.00DL	11.50	18.80	0.20DL	9.45	0.20DL	0.20DL	NS
	24	137.0	24.60	0.20DL	206.00	0.20DL	0.200DL	0.53	12.40	15.30	1.98	0.20DL	10.60	0.20DL	0.20DL	2.50DL
MW104U	1	10,500.0	23,400.0	1,530.0	442.00	0.20DL	0.200DL	165.00	2.00DL	9,110.0	519.0	0.20DL	0.20DL	NS	NS	NS
	2	11,300.0	24,200.0	1,830.0	345.00	86.3	0.200DL	108.00	2.00DL	9,840.0	537.0	4.030	16.80	NS	NS	NS
	3	13,900.0	24,000.0	1,840.0	374.00	0.20DL	0.200DL	116.00	2.00DL	9,540.0	450.0	0.20DL	0.20DL	NS	NS	NS
	4	15,100.0	22,610.0	1,780.0	512.00	0.20DL	0.200DL	108.00	2.00DL	8,720.0	527.0	0.20DL	0.20DL	NS	NS	NS
	5	11,500.0	25,300.0	1,860.0	453.00	0.20DL	0.200DL	133.00	2.00DL	9,850.0	497.0	0.20DL	0.20DL	NS	NS	NS
	6	11,300.0	21,700.0	1,890.0	442.00	0.20DL	0.200DL	132.00	2.00DL	9,570.0	559.0	0.20DL	17.00	NS	NS	NS
	10	10,900.0	22,300.0	1,690.0	392.00	0.20DL	0.200DL	125.00	2.00DL	9,280.0	526.0	0.20DL	0.20DL	NS	NS	NS
	13	10,800.0	21,800.0	1,790.0	332.00	0.20DL	0.200DL	131.00	2.00DL	9,100.0	403.0	0.20DL	0.20DL	0.20DL	0.20DL	NS

Appendix Table 2 (Continued)

Well	Sampling Month[1]	TNT	RDX	HMX	2,4DNT	2,6DNT	4ADNT	2ADNT	2,4DANT	TNB	DNB	NB	3,5DNA	MNX	TNX	MNHMX
MW104U (Cont.)	16	13,800.0	21,400.0	1,770.0	376.00	0.20DL	0.200DL	125.00	2.00DL	9,500.0	435.0	0.20DL	28.10	0.20DL	0.20DL	NS
	19	10,100.0	25,500.0	1,840.0	389.00	0.20DL	0.200DL	109.00	2.00DL	9,200.0	461.0	0.20DL	10.90	0.20DL	0.20DL	NS
	21	10,500.0	22,700.0	1,923.0	363.00	0.20DL	0.200DL	126.00	2.00DL	9,810.0	420.0	0.20DL	9.35	0.20DL	0.20DL	NS
	24	10,300.0	20,900.0	1,800.0	398.0	0.20DL	0.200DL	119.00	22.10	9,130.0	453.0	0.20DL	12.20	20.40	0.20DL	33.20
MW105L	1	390.0	1,430.0	116.0	27.0	0.20DL	2.250	6.690	2.00DL	649.0	49.50	0.20DL	11.90	NS	NS	NS
	2	444.00	1,120.0	107.0	17.40	0.20DL	0.200DL	5.250	2.00DL	535.0	44.0	0.20DL	0.20DL	NS	NS	NS
	3	242.0	882.0	80.70	16.00	5.330	1.530	5.100	2.00DL	434.0	35.80	0.20DL	0.20DL	NS	NS	NS
	4	178.0	683.0	63.70	2.980	0.20DL	1.540	5.100	2.00DL	407.0	24.10	0.20DL	0.20DL	NS	NS	NS
	5	129.0	532.0	48.60	13.10	0.20DL	1.190	3.190	2.00DL	254.0	2.450	0.20DL	0.20DL	NS	NS	NS
	6	111.0	471.0	28.40	11.60	0.20DL	1.930	2.870	2.00DL	199.0	22.20	0.20DL	0.20DL	NS	NS	NS
	10	132.0	554.0	36.80	14.10	0.20DL	0.200DL	3.210	5.630	192.0	22.70	0.20DL	2.250	NS	NS	NS
	13	84.20	388.0	22.30	9.73	0.20DL	0.200DL	1.90	2.00DL	137.0	17.70	0.20DL	0.81	0.20DL	0.20DL	NS
	16	124.0	470.0	29.90	14.40	0.20DL	0.200DL	4.39	2.00DL	176.0	19.50	0.20DL	1.01	0.20DL	0.20DL	NS
	19	177.0	654.0	10.40	7.59	0.20DL	0.200DL	2.26	2.00DL	158.0	9.70	0.20DL	1.64	0.20DL	0.20DL	NS
	21	176.0	707.0	37.30	14.70	0.20DL	0.200DL	0.20DL	2.00DL	181.0	30.20	0.20DL	0.91	0.20DL	0.20DL	NS
	24	87.30	356.0	15.70	8.05	0.20DL	0.200DL	0.96	4.18	96.0	14.40	0.20DL	1.20	2.38	0.20DL	4.71
MW107L	1	0.190J	6.560	0.560	0.440	0.20DL	0.780	0.200	2.00DL	0.850	0.210	0.890	0.540	NS	NS	NS
	2	0.20DL	5.920	0.20DL	0.20DL	0.20DL	0.780	0.200	2.00DL	0.870	0.200	0.490	0.20DL	NS	NS	NS
	3	0.20DL	8.240	0.20DL	0.690	0.20DL	0.990	0.180J	0.840J	0.670	0.210	0.590	0.340	NS	NS	NS
	4	0.20DL	8.910	0.20DL	0.690	0.20DL	1.040	0.240	1.270	1.040	0.200	1.560	0.790	NS	NS	NS
	5	0.20DL	9.570	0.20DL	0.20DL	0.20DL	0.730	0.150J	0.390J	0.750	0.230	0.560	1.510	NS	NS	NS

Appendix Table 2 (Continued)

Well	Sampling Month[1]	TNT	RDX	HMX	2,4DNT	2,6DNT	4ADNT	2ADNT	2,4DANT	TNB	DNB	NB	3,5DNA	MNX	TNX	MNHMX
MW107L (Cont.)	6	0.20DL	9.440	0.20DL	0.20DL	0.20DL	1.140	0.20DL	2.00DL	0.550	0.220	0.20DL	0.20DL	NS	NS	NS
	10	0.20DL	8.180	0.20DL	0.920	0.20DL	1.130	0.20DL	2.00DL	0.580	0.220	0.20DL	0.50	NS	NS	NS
	13	0.20DL	8.16	0.20DL	0.71	0.20DL	1.09	0.20DL	2.00DL	0.53	0.18DL	0.20DL	0.20DL	0.20DL	0.20DL	NS
	24	0.20DL	7.81	0.20DL	0.72	0.20DL	0.18J	0.20DL	56.00	0.33	0.21	6.7	0.15J	0.20DL	0.20DL	2.50DL
MW108L	10	706.0	12.20	0.20DL	90.0	0.20DL	0.20DL	0.630	0.20DL	912.0	14.40	0.20DL	0.20DL	NS	NS	NS
	13	578.0	10.80	0.20DL	73.2	0.20DL	0.20DL	1.00	0.20DL	846.0	9.94	0.20DL	0.20DL	0.20DL	0.20DL	NS
	16	642.0	9.40	1.16	77.80	0.20DL	0.20DL	1.22	0.20DL	930.0	9.39	0.20DL	0.20DL	0.20DL	0.20DL	NS
	19	564.0	7.97	0.20DL	71.40	0.20DL	0.20DL	0.20DL	2.00DL	870.0	9.05	0.20DL	0.69	0.20DL	0.20DL	NS
	21	543.0	8.69	0.20DL	73.30	0.20DL	0.20DL	0.83	2.00DL	899.0	10.50	0.20DL	0.40	0.20DL	0.20DL	NS
	24	477.0	8.26	1.05	67.20	0.20DL	0.20DL	0.13J	1.43J	844.0	9.52	0.20DL	0.36	0.30	0.20DL	2.50DL
MW109U	1	2,940.0	3,950.0	674.0	114.0	0.20DL	116.00	195.0	22.30	94.90	8.350	0.20DL	340.0	NS	NS	NS
	2	3,050.0	4,330.0	692.0	73.0	44.50	117.00	184.0	18.80	80.0	4.570	0.20DL	249.0	NS	NS	NS
	3	3,230.0	4,560.0	556.0	84.20	0.20DL	138.0	198.0	51.10	74.60	4.990	0.20DL	227.0	NS	NS	NS
	4	2,780.0	3,960.0	527.0	91.40	0.20DL	115.0	169.0	48.70	76.80	4.580	0.20DL	263.0	NS	NS	NS
	5	3,360.0	4,700.0	583.0	96.50	0.20DL	133.0	195.0	24.40	87.70	5.00	0.20DL	311.0	NS	NS	NS
	6	2,820.0	4,650.0	622.0	90.40	0.20DL	187.0	217.0	13.20	85.60	5.360	0.20DL	296.0	NS	NS	NS
	10	2,960.0	4,340.0	655.0	92.0	0.20DL	357.0	190.0	40.80	98.80	6.890	0.20DL	292.0	NS	NS	NS
	13	2,820.0	4,090.0	650.0	59.20	0.20DL	132.0	188.0	2.00DL	69.40	5.92	0.20DL	206.0	0.20DL	0.20DL	NS
	16	1,540.0	3,580.0	639.0	38.60	0.20DL	156.0	177.0	2.00DL	48.70	7.11	0.20DL	63.30	0.20DL	0.20DL	NS
	19	1,440.0	4,270.0	703.0	38.60	0.20DL	152.0	189.0	2.00DL	47.10	7.47	0.20DL	97.0	0.20DL	0.20DL	NS
	21	1,987.0	4,619.0	642.0	57.80	0.20DL	168.0	173.0	2.00DL	54.30	11.40	0.20DL	134.0	0.20DL	0.20DL	NS

Appendix Table 2 (Continued)

Well	Sampling Month[1]	TNT	RDX	HMX	2,4DNT	2,6DNT	4ADNT	2ADNT	2,4DANT	TNB	DNB	NB	3,5DNA	MNX	TNX	MNHMX
MW109U (Cont.)	24	1,700.0	4,720.0	631.0	44.90	0.20DL	146.0	189.0	17.10	46.70	6.78	0.20DL	93.40	1.74	22.40	16.10
MW110L	1	664.0	3,650.0	217.0	62.20	0.20DL	74.50	107.0	57.70	471.0	23.60	0.20DL	125.0	NS	NS	NS
	2	694.0	4,200.0	247.0	53.40	0.20DL	69.0	90.60	43.40	444.0	22.80	0.20DL	100.0	NS	NS	NS
	3	715.0	4,240.0	222.0	58.10	0.20DL	67.70	70.40	133.0	499.0	24.50	0.20DL	64.60	NS	NS	NS
	4	729.0	4,060.0	224.0	64.90	0.20DL	63.0	58.80	151.0	603.0	25.20	0.20DL	68.80	NS	NS	NS
	5	853.0	4,900.0	291.0	68.50	0.20DL	70.40	62.30	77.20	658.0	28.0	0.20DL	71.80	NS	NS	NS
	6	787.0	4,260.0	285.0	65.50	0.20DL	83.70	70.0	38.60	626.0	27.10	0.20DL	86.00	NS	NS	NS
	10	782.0	3,870.0	272.0	65.0	0.20DL	69.40	59.80	33.0	571.0	24.80	0.20DL	68.0	NS	NS	NS
	13	624.0	4,030.0	243.0	50.40	0.20DL	69.40	65.40	2.0DL	460.0	20.20	0.20DL	59.10	0.20DL	0.20DL	NS
	16	881.0	3,760.0	212.0	58.50	0.20DL	61.30	55.90	2.0DL	518.0	22.90	0.20DL	60.60	0.20DL	0.20DL	NS
	19	775.0	4,400.0	264.0	58.80	0.20DL	56.10	57.20	2.0DL	566.0	22.60	0.20DL	74.60	0.20DL	0.20DL	NS
	21	753.0	3,956.0	226.0	56.20	0.20DL	62.90	62.30	2.0DL	507.0	21.30	0.20DL	66.50	0.20DL	0.20DL	NS
	24	710.0	3,860.0	240.0	54.60	0.20DL	57.0	63.20	12.30	491.0	21.30	0.20DL	90.30	12.20	76.80	5.19
MW112L	1	152.0	640.0	19.20	10.50	0.20DL	1.080	1.440	3.480	15.60	10.20	0.20DL	9.350	NS	NS	NS
	2	60.20	319.0	8.570	4.140	0.20DL	0.910	1.400	1.430J	11.60	4.370	0.20DL	0.20DL	NS	NS	NS
	3	92.0	395.0	7.570	5.820	0.20DL	0.990	1.280	4.120	6.980	5.540	0.20DL	0.20DL	NS	NS	NS
	4	110.0	326.0	7.980	0.800	0.20DL	1.130	1.320	6.20	13.50	6.330	0.20DL	0.20DL	NS	NS	NS
	5	134.0	548.0	14.60	9.120	0.20DL	1.730	1.870	3.260	10.70	7.660	0.20DL	0.20DL	NS	NS	NS
	6	112.0	382.0	9.080	7.940	0.20DL	0.20DL	1.260	1.530J	7.490	6.670	0.20DL	0.20DL	NS	NS	NS
	10	96.90	349.0	7.790	10.30	0.20DL	1.220	1.320	2.190	6.730	6.260	0.20DL	0.20DL	NS	NS	NS
	13	71.60	304.0	6.24	5.40	0.20DL	1.03	1.02	2.00DL	4.66	4.25	0.20DL	0.20DL	0.20DL	0.20DL	NS

Appendix Table 2 (Continued)

Well	Sampling Month[1]	TNT	RDX	HMX	2,4DNT	2,6DNT	4ADNT	2ADNT	2,4DANT	TNB	DNB	NB	3,5DNA	MNX	TNX	MNHMX
MW112L (Cont.)	16	127.0	462.0	8.99	9.54	0.20DL	1.45	1.39	2.00DL	5.98	7.16	0.20DL	0.20DL	0.20DL	0.20DL	NS
	19	130.0	610.0	12.00	9.05	0.20DL	0.64	0.77	2.00DL	6.40	6.83	0.20DL	0.20DL	0.20DL	0.20DL	NS
	21	111.0	522.0	10.60	8.95	0.20DL	1.19	0.90	2.00DL	5.36	7.49	0.20DL	2.62	0.20DL	0.20DL	NS
	24	71.20	350.0	66.40	6.53	0.20DL	0.82	0.82	1.12J	6.07	5.69	0.20DL	1.35	0.20DL	2.69	0.31J
MW140U	1	2,430.0	3,710.0	182.0	106.0	0.20DL	14.30	22.40	2.00DL	22.30	84.50	0.20DL	0.20DL	NS	NS	NS
	2	2,630.0	4,200.0	209.0	108.0	0.20DL	18.0	22.80	2.00DL	29.20	88.30	0.20DL	7.180	NS	NS	NS
	3	3,120.0	4,140.0	162.0	101.0	0.20DL	15.0	23.80	17.20	13.70	82.0	0.20DL	6.510	NS	NS	NS
	4	2,930.0	3,900.0	156.0	103.0	0.20DL	16.60	20.70	17.90	18.50	77.20	0.20DL	5.480	NS	NS	NS
	5	2,800.0	4,490.0	197.0	116.0	0.20DL	21.10	27.00	9.720	21.60	88.20	0.20DL	8.380	NS	NS	NS
	6	2,510.0	4,010.0	213.0	106.0	0.20DL	32.60	25.70	4.910	19.40	84.50	0.20DL	5.620	NS	NS	NS
	10	2,360.0	3,420.0	184.0	91.0	0.20DL	14.60	20.20	7.620	11.80	78.50	0.20DL	5.980	NS	NS	NS
	13	2,070.0	2,980.0	149.0	50.40	0.20DL	19.40	25.60	2.00DL	13.20	54.60	0.20DL	3.82	0.20DL	0.20DL	NS
	16	2,030.0	2,780.0	137.0	83.60	0.20DL	19.20	22.20	2.00DL	12.80	63.0	0.20DL	5.70	0.20DL	0.20DL	NS
	19	1,780.0	3,030.0	132.0	78.10	0.20DL	13.0	18.80	2.00DL	11.10	56.20	0.20DL	5.12	0.20DL	0.20DL	NS
	21	1,889.0	2,666.0	132.0	80.80	0.20DL	15.20	18.50	2.00DL	11.10	58.70	0.20DL	4.14	0.20DL	0.20DL	NS
	24	1,880.0	2,570.0	138.0	87.80	0.20DL	15.30	16.50	3.97	14.30	63.0	0.20DL	6.37	0.16J	13.20	2.50DL
MW141L	1	2,090.0	840.0	10.20	390.0	0.20DL	0.20DL	5.80	2.00DL	1,260.0	95.70	12.0	0.20DL	NS	NS	NS
	2	2,810.0	933.0	0.20DL	456.0	0.20DL	0.20DL	0.550	2.00DL	1,600.0	120.0	0.20DL	10.30	NS	NS	NS
	3	3,420.0	836.0	2.720	410.0	0.20DL	0.20DL	2.820	2.00DL	1,500.0	98.30	0.20DL	4.50	NS	NS	NS
	4	2,640.0	1,040.0	52.10	498.0	0.20DL	0.20DL	5.680	2.00DL	1,420.0	120.0	0.20DL	11.20	NS	NS	NS
	5	3,100.0	1,120.0	14.80	560.0	0.20DL	0.20DL	2.990	2.00DL	1,840.0	136.0	0.20DL	10.30	NS	NS	NS

Appendix Table 2 (Concluded)

Well	Sampling Month[1]	TNT	RDX	HMX	2,4DNT	2,6DNT	4ADNT	2ADNT	2,4DANT	TNB	DNB	NB	3,5DNA	MNX	TNX	MNHMX
MW141L (Cont.)	6	2,770.0	1,100.0	14.00	535.0	0.20DL	0.20DL	0.20DL	2.00DL	1,680.0	134.0	0.20DL	7.170	NS	NS	NS
	10	2,460.0	965.0	11.00	417.0	0.20DL	0.20DL	1.770	2.00DL	1,450.0	112.0	0.20DL	4.730	NS	NS	NS
	13	2,500.0	1,120.0	11.70	366.0	0.20DL	0.20DL	5.29	2.00DL	1,520.0	97.40	0.20DL	4.79	0.20DL	0.20DL	NS
	16	2,650.0	1,200.0	14.50	466.0	0.20DL	0.20DL	5.12	2.00DL	1,720.0	119.0	0.20DL	6.93	0.20DL	0.20DL	NS
	19	2,560.0	1,420.0	16.70	461.0	0.20DL	0.20DL	1.64	2.00DL	1,620.0	124.0	0.20DL	6.59	0.20DL	0.20DL	NS
	21	2,575.0	1,222.0	12.60	453.0	0.20DL	0.20DL	0.20DL	2.00DL	1,591.0	123.0	0.20DL	4.25	0.20DL	0.20DL	NS
	24	2,680.0	1,330.0	14.80	473.0	0.20DL	0.20DL	3.18	9.17	1,670.0	127.0	0.20DL	4.81	1.18	0.20DL	2.50DL
MW168L	1	1,720.0	425.0	5.150	430.0	0.20DL	0.20DL	0.20DL	2.00DL	209.0	104.0	0.20DL	0.20DL	NS	NS	NS
	2	2,520.0	386.0	0.20DL	404.0	0.20DL	0.20DL	0.540	2.00DL	223.0	114.0	0.20DL	5.740	NS	NS	NS
	3	2,350.0	358.0	1.550	367.0	0.20DL	0.20DL	1.530	2.560	144.0	88.40	0.20DL	5.040	NS	NS	NS
	4	1,780.0	400.0	3.140	432.0	0.20DL	0.20DL	1.710	2.0DL	191.0	102.0	0.20DL	7.480	NS	NS	NS
	5	1,790.0	436.0	4.870	392.0	0.20DL	0.20DL	1.440	2.0DL	175.0	103.0	0.20DL	5.490	NS	NS	NS
	6	1,920.0	484.0	4.010	490.0	0.20DL	0.20DL	0.20DL	2.0DL	213.0	121.0	0.20DL	4.290	NS	NS	NS
	10	1,890.0	455.0	3.150	414.0	0.20DL	0.20DL	3.930	2.0DL	221.0	110.0	0.20DL	4.050	NS	NS	NS
	13	2,000.0	496.0	3.74	370.0	0.20DL	0.20DL	0.20DL	2.0DL	174.0	97.20	0.20DL	3.600	0.20DL	0.20DL	NS
	16	1,990.0	503.0	7.46	476.0	0.20DL	0.20DL	0.20DL	2.0DL	189.0	119.0	0.20DL	7.11	0.20DL	0.20DL	NS
	19	1,980.0	685.0	3.74	456.0	0.20DL	0.20DL	0.20DL	2.0DL	208.0	119.0	0.20DL	4.58	0.20DL	0.20DL	NS
	21	2,050.0	540.0	4.38	450.0	0.20DL	0.20DL	0.20DL	2.0DL	199.0	116.0	0.20DL	3.27	0.20DL	0.20DL	NS
	24	1,960.0	540.0	4.88	452.0	0.20DL	0.20DL	3.85	2.0DL	201.0	118.0	0.20DL	4.47	0.20DL	0.20DL	2.50DL

3 Site-Capacity Estimation Parameters

Introduction

Two key estimates of site capacity for explosives can be obtained by measuring adsorption and disappearance of explosives which are due to multiple and complex processes in the soil and aquifer. Adsorption is a nondestructive reduction in groundwater concentrations of explosives because of interactions with the soil that are sometimes reversible. Contaminant disappearance may be due to the interactions of complex processes including biodegradation, abiotic transformation, covalent bonding to the substrate, and adsorption. Pseudo-first-order kinetics, which indicate the half-life of the contaminant of interest, and adsorption coefficients, which indicate the affinity of site soil for the contaminant and the contaminant leaching potential, can be used to refine groundwater model input parameters. The objective of this study was to obtain site-specific sorption coefficients and disappearance rates for explosives in LAAP aquifer soils.

Materials and Methods

Soil collection

Based on examination of geophysical information from existing boring logs for boreholes in proximity to Area P, four main soil types were identified in the LAAP aquifer. An area north of Area P containing a borrow pit was identified as a source of clean soils that contained the four main aquifer soil types, an ML silt, SP-SM sandy silt, SM silty sand, and CL lean clay according to the Unified Soil Classification System (U.S. Army Corps of Engineers 1960). These soils were obtained by trenching with a backhoe at depths of 4 to 7.5 ft below ground surface (CL), 7.5 to 11.5 ft below ground surface (SM), 8 to 11 ft below ground surface (ML), and 12 to 15 ft below ground surface (SP-SM). Soil samples were stored in 5-gal plastic buckets, sealed, and refrigerated at 4 °C until used. Physical and chemical characteristics of the soils were determined as described for samples collected by cone penetrometry (Chapter 2).

Groundwater collection

Contaminated groundwater from MW85U and uncontaminated groundwater from MW70U were collected immediately following field sampling of the wells during monitoring. The anaerobic well water was pumped into 4-L precleaned glass bottles to the point of overflow, sealed with Teflon-coated lids, then refrigerated at 4 °C until used. Water from MW85U contained high concentrations of all of the explosives contaminants observed in LAAP groundwater. Chemical analysis results for MW85U and MW70U can be found in Chapter 2, Groundwater Monitoring and Cone Penetrometry.

Batch testing

Kinetic. The four aquifer soils from LAAP were tested under a nitrogen atmosphere. Polycarbonate centrifuge tubes (500-mL, cleaned in acid) were loaded in triplicate with 90-g oven-dried weight (ODW) of each of the aquifer soils and sufficient water from LAAP MW85U to bring water weight to 360 g. The tubes were sealed, placed in a rotary shaker at 20 revolutions per minute, and sampled at intervals of 0.5, 1, 6, 24, 48, 72, 96, and 168 hr. At each sampling time, a 10-mL subsample of the slurry was placed into a 25-mL centrifuge tube and spun at a relative centrifugal force (RCF) of 10,000 for 20 min. The aqueous phase was separated, brought to a final concentration of 5 μM of ethylenediaminetraacetate (EDTA) and analyzed by HPLC (U.S. Environmental Protection Agency 1990). At the conclusion of the test, a soil sample was also analyzed. All operations were conducted at 20 °C and, where practicable, in the dark to avoid photodegradation. Solutions were analyzed for TNT, TNB, DNB, 4ADNT, 2ADNT, 2,4DANT, RDX, HMX, picric acid, 2,4DNT, and 2,6DNT according to methods described in Chapter 2.

Equilibrium. Adsorption isotherm tests were conducted at 20 °C under a nitrogen atmosphere in duplicate with 25-mL centrifuge tubes containing 4 g ODW of each of the four aquifer soils and 16 mL of well water. Water from MW85U was used full strength and diluted to 0.85, 0.70, 0.5, and 0.3 percent of full strength with contaminant-free water from MW70U. The tubes were sealed and placed in a rotary mixer at 20 revolutions per minute, sampled after 24 hr, the time required to reach steady-state HMX, RDX, DNB, 2ADNT, and 2,4DNT solution concentrations,[1] and centrifuged at 10,000 RCF for 20 min. Five milliliters of the aqueous phase was removed, preserved with EDTA, and frozen until analyzed by HPLC for the same parameters and by the same methods as in the kinetic studies.

Column testing

Soil column breakthrough curves (BTCs). Soil column experiments were conducted with the four soils in stainless steel columns 15.24 cm in length, with a

[1] TNT never reached steady-state solution concentration; therefore, TNT equilibration time was based upon time required to achieve steady state by other analytes.

4.45-cm ID. Soils were loaded into columns in two approximately equal lifts. The soil surface was scarified between lifts to minimize bedding plane formation. Flows (upflow mode) were set to provide average pore-water velocities of 10^{-4} cm sec^{-1} using constant-volume metering pumps.

After loading, uncontaminated groundwater from MW70U was pumped at steady flow through the columns for approximately 3 weeks in order to allow the hydraulic properties of the columns to stabilize. Groundwater from MW85U was then pumped into the columns at steady flow to provide a step input loading sufficient to displace approximately 9 to 13 pore volumes. Concentrations of the same parameters measured in the batch testing were monitored throughout the experiment. After step input loading, columns were eluted with uncontaminated groundwater from MW70U at the same flow used for the step input loading. Column eluate samples were collected throughout the experiment in approximately 15-mL increments in amber glass vials (20-mL) using automated fraction collectors. Aliquots (5-mL) of each eluate sample were preserved with an equal amount of acetonitrile within 24 hr of collection and stored at 4 °C in capped vials until analyzed.

Parameter estimation. Pseudo-first-order disappearance rate constants (μ) and equilibrium sorption coefficients (linear (K_D), Freundlich (K_f), and Langmuir (K_L)) were obtained by fitting a semianalytical model to TNT breakthrough curves (Myers, Townsend, and Hill 1998).

Analytical methods

Concentrations of explosives and TNT transformation products were analyzed by HPLC as described in Chapter 2, Groundwater Monitoring and Cone Penetrometry. Total organic carbon (TOC) was determined by the American Public Health Association (APHA) (1989) Method 5301D. Soil pH was determined on magnetically stirred soil slurries (1:1, soil:distilled deionized water) using a Beckman Model SS-3 pH meter (Beckman Instruments, Inc., Fullerton, CA) (U.S. Environmental Protection Agency 1986). Cation exchange capacity (CEC) was determined by the ammonium saturation method (Plumb 1981). Particle-size distribution was determined on air-dried soil using the method of Day (1956) as modified by Patrick (1958). Permeability was measured using the falling head method (MacIver and Hale 1970).

Results and Discussion

Soil properties

Aquifer soils from LAAP were generally high in sand, ranging from 65- to 92.5-percent sand (Table 1). Silt and clay was present in all samples, although in lower amounts. Total organic carbon content was low, ranging from 0.015 to 0.162 percent. Cation exchange capacity was also low, ranging from 3.5 to 8.1 Meq 100 g^{-1}. Soil pH was acidic and relatively consistent for all soil types

Table 1
Physical Characteristics of LAAP Aquifer Soils

Soil	Sand, %	Silt, %	Clay, %	CEC Me 100 g^{-1}	pH	Total Organic Carbon, %	Permeability cm sec^{-1}
CL lean clay	65	20	15	8.1	5.5	0.162	7.75×10^{-7}
ML sandy silt	90	5	5	3.5	5.6	0.015	3.17×10^{-4}
SM silty sand	85	7.5	7.5	5.5	5.5	0.02	$>10^{-9}$
SP-SM sandy silt	92.5	2.5	5	3.6	5.6	0.015	7.75×10^{-7}

(average of 5.55, Table 1). Permeability of the soils ranged from 10^{-4} to $>10^{-9}$ cm sec^{-1}.

Batch testing

Adsorption kinetics. Concentrations of TNT in the solution phase decreased slowly following exposure to LAAP aquifer soils (Figure 1). Exposure to the soil containing the highest clay and organic carbon (CL soil) resulted in the lowest solution concentration. Other explosives compounds were also relatively stable or declined slightly over time (Figure 2 and Chapter 3 Appendix). The lowest recovery of explosives from solution was observed for DNB in all the aquifer soils, but especially in the SM silty sand and CL lean clay (Figure 3). These results imply that DNB was more degradable than the other contaminants.

The rates of processes that remove explosives contaminants from solution can be modeled using pseudo-first-order kinetics that take the form

$$dc / dt = -kc \tag{1}$$

where

c = chemical concentration of reacting substance, mg L^{-1}

k = pseudo-first-order reaction constant, hr^{-1}

t = time, hr

Pseudo-first-order kinetics reduces to the equation

$$ln(c_0 / c) = kt \tag{2}$$

where c_0 is the concentration of the reacting substance at time 0. Once a value of k is obtained, the half-life period of the reacting substance, $t_{1/2}$ can be calculated using the equation

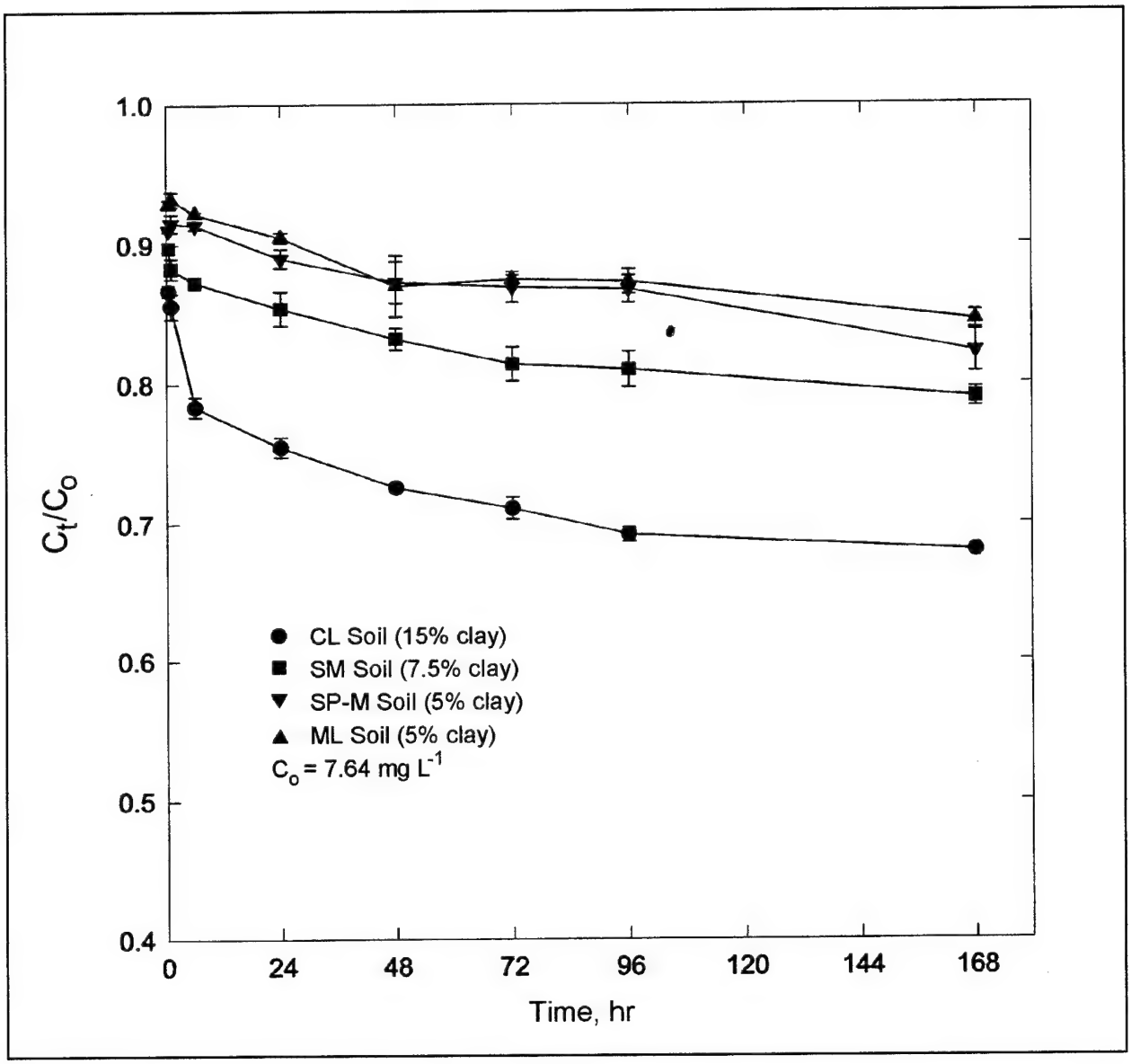

Figure 1. Adsoprtion kinetics of TNT (C_o = 7.645 mg/L) from LAAP groundwater (MW85U) under anaerobic conditions in LAAP aquifer soils

$$t_{1/2} = 0.693 / k \tag{3}$$

To quantify the rates of disappearance, concentrations of explosives in the test were fit to the pseudo-first-order kinetic equation. Analysis of the kinetic data showed that the solution concentrations could be modeled using pseudo-first-order kinetics (Table 2) with the strongest correlations for TNT, TNB, and 2,4DNT. First-order rate coefficients varied between aquifer soils and compounds, but were generally low, with the highest measured coefficient of 0.017 hr^{-1} observed for 2,4DNT in the ML soil. Most of the rate coefficients were not significantly different from, or approached, zero. The removal-rate constants and half-lives for

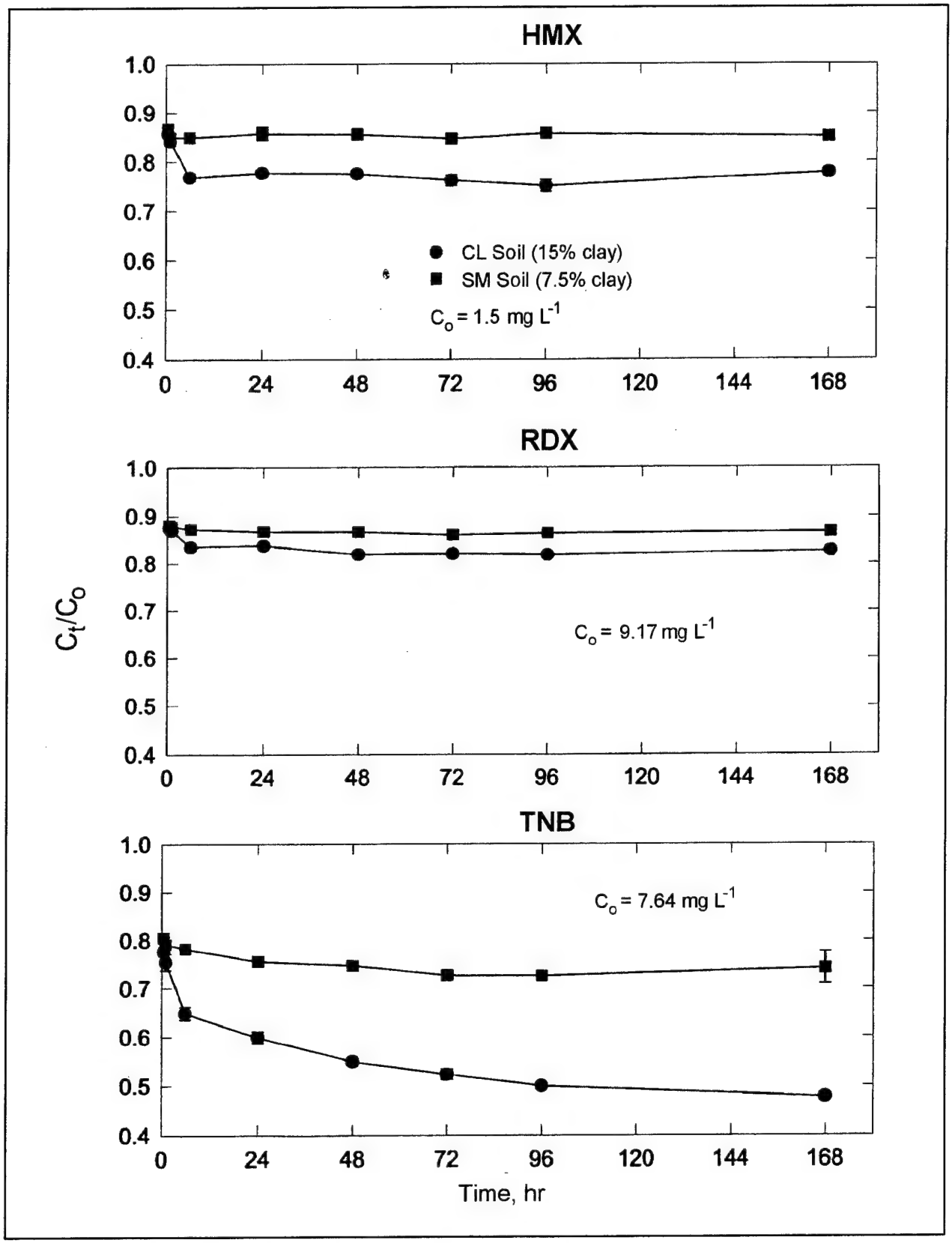

Figure 2. Adsoprtion of HMX, RDX, and TNB by LAAP aquifer soils

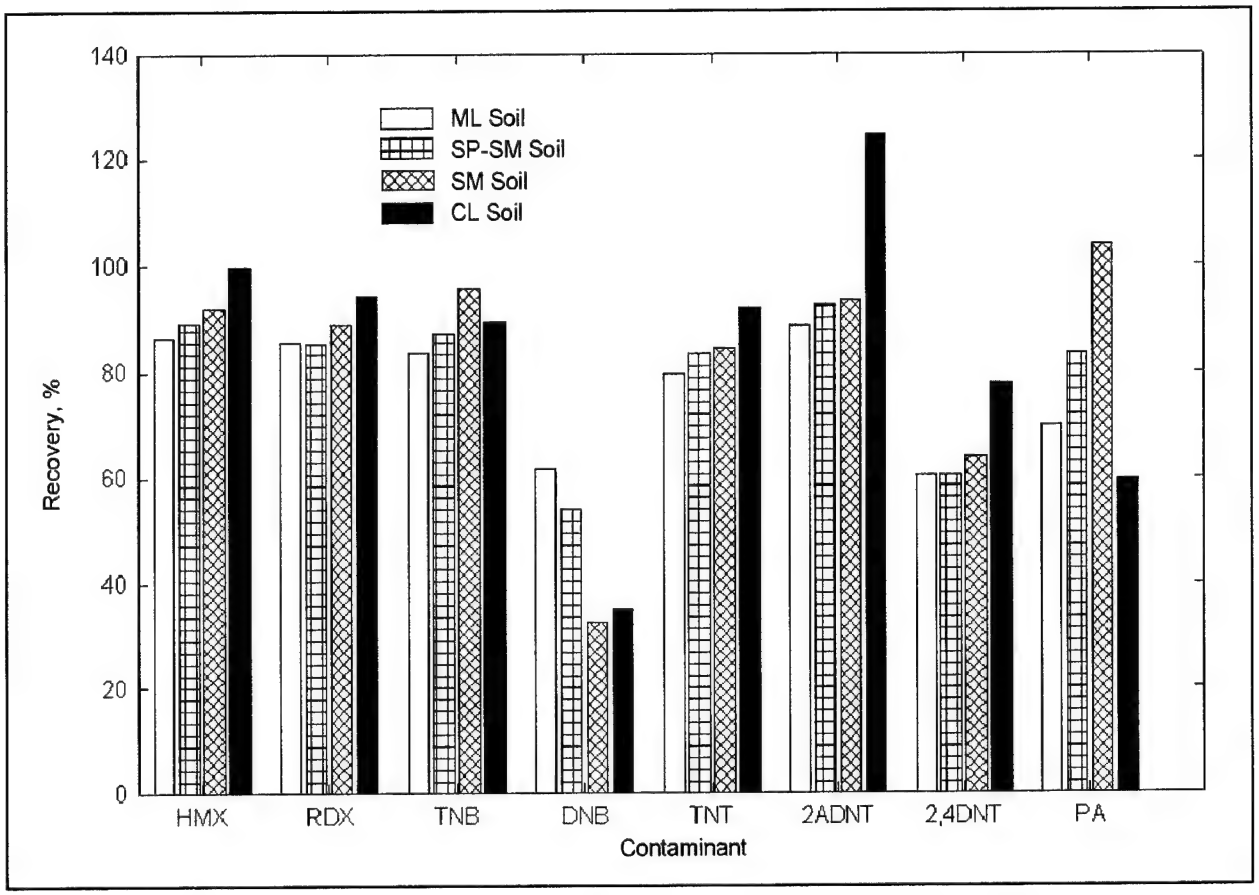

Figure 3. Percent recovery of soil + solution explosives contaminants following exposure of LAAP groundwater to LAAP aquifer soils for 168 hr

explosives contaminants in the LAAP aquifer soils were low compared with those observed for surface soils (Brannon and Myers 1997).

Adsorption capacity. Adsorption of explosives from groundwater by the LAAP aquifer soils was limited (Table 3). The use of multicomponent water in testing accounts for competitive interactions between explosives at the ratios tested. However, experiments with single-component explosives yielded similar partitioning results as multicomponent systems, implying that competitive adsorption was not a significant factor. The data for HMX, RDX, and TNB fit the following linear adsorption model reasonably well:

$$K_D = q_{SOIL} / C_W \tag{4}$$

where

K_D = distribution coefficient, L kg^{-1}

q_{SOIL} = concentration of contaminant sorbed to aquifer soil, mg k^{-1}

Table 2
First-Order Rate Coefficients (k, hr^{-1}, Regression Coefficients (r^2), and Half-Lives ($t^{1/2}$, hr) for Explosives in Four LAAP Aquifer Soils Under Anaerobic Conditions (Solution phase consisted of undiluted contaminated water from MW85U)

	CL Lean Clay			ML Silt			SM Silty Sand			SP-SM Sandy Silt		
Compound	k	r^2	$t_{1/2}$	k	r^2	$t_{1/2}$	k	r^2	$t_{1/2}$	k	r^2	$t_{1/2}$
HMX	0.00044	0.26	1,580	NS[1]	0.06	NS	NS	0.02	NS	NS	0.03	NS
RDX	0.0003	0.39	2,380	NS	0.01	NS	0.00009	0.27	7,360	NS	0.0001	NS
TNB	0.0027	0.74	250	0.0006	0.83	1,230	0.0005	0.38	1,390	0.0006	0.63	1,220
DNB	0.0013	0.53	550	NS	0.13	NS	0.0019	0.61	360	0.0019	0.64	370
TNT	0.0014	0.72	510	0.0006	0.70	1,220	0.0007	0.78	940	0.0006	0.83	1,120
2ADNT	0.0011	0.44	630	0.0004	0.44	1,890	0.0005	0.23	1,270	0.0003	0.28	2,480
2,4DNT	0.0021	0.75	330	0.017	0.75	40	0.0021	0.56	320	0.0017	0.68	410
Picric acid	0.0037	0.60	190	NS	0.17	NS	NS	0.00	NS	NS	0.003	NS

[1] NS = Rate constant is not significantly ($p < 0.5$) different from zero.

C_W = aqueous phase contaminant concentration, mg L^{-1}

Fifteen of the twenty-five instances where adsorption was observed resulted in r^2 values of 0.7 or greater. In the other 10 instances where r^2 values were below 0.7, the analysis of variance conducted for each regression showed that these relationships were statistically significant ($p < 0.05$) (Table 3).

The measured values of K_D were below 1 L kg^{-1} for all soils and compounds tested, ranging from no significant adsorption to a high value of 0.84 L kg^{-1}. The highest degree of sorption was associated with the CL and SM soils, the two soils with the highest clay content and CEC. These values are generally lower than previously reported explosives sorption coefficients in a wide variety of soils (Townsend and Myers 1996). The range of sorption coefficients between soils varied over an order of magnitude for explosive compounds. For modeling of contaminant transport at the LAAP site, the use of an average value of K_D to represent sorption at the site was appropriate. These data indicated that retardation of the contaminant plume by any of the major soil types present in the aquifer is limited by the low degree of adsorption of explosives. However, other factors, such as limited permeability of some soil types, may have more impact on movement of the plume than the intrinsic sorption and transformation potential of the aquifer soils.

Column testing

Soil column BTCs. TNT BTCs followed characteristic patterns (Figure 4). The TNT BTCs for the ML and SP-SM soils were approximately symmetrical.

Table 3
Distribution Coefficients (K_D, L kg^{-1}) and Regression Coefficients (r^2) for Explosives in Four LAAP Aquifer Soils Under Anaerobic Conditions

	CL Soil		ML Soil		SM Soil		SP-SM Soil	
Compound	K_D	r^2	K_D	r^2	K_D	r^2	K_D	r^2
HMX	0.37	0.94	0.086	0.73	0.20	0.77	0.20	0.81
RDX	0.33	0.83	0.21	0.95	0.33	0.95	0.33	0.97
TNB	0.49	0.99	0.16	0.92	0.27	0.88	0.21	0.86
DNB	0.32	0.59	NSA[1]		NSA		NSA	
TNT	0.27	0.92	0.04	0.34	0.17	0.68	0.09	0.45
2,4DNT	0.67	0.85	0.09	0.30	NSA		0.28	0.42
3,5DNA	0.84	0.66	0.28	0.71	0.37	0.51	0.21	0.52
Picric acid	0.41	0.53	NSA		NSA		NSA	

[1] NSA = No significant adsorption.

The TNT BTCs for the CL and SM soils were slightly asymmetrical because of tailing near the end of the curves, suggesting nonlinear or nonequilibrium sorption. The BTCs for the SP-SM and CL soils showed steady-state TNT concentrations that approximated the TNT input concentration, whereas steady-state TNT concentrations were slightly lower than the input concentrations for the ML and SM soils.

Most of the TNT (96-105 percent) introduced to the soil columns could be accounted for in the mass balance. TNT concentrations measured in the soils after completion of the BTCs were less than detection limits (0.100 mg kg^{-1}) in each soil except for the CL soil with the highest clay content, which contained TNT concentrations of 0.200 mg kg^{-1}, which was just above the detection limits.

Parameter estimation. Adsorption coefficients and uptake rate coefficients (μ) for TNT were obtained from fitting the Langmuir, Freundlich, and linear adsorption models to the observed column data (Table 4). The Langmuir adsorption model was a marginally better fit than the linear model in three of the four soils based on comparison of root mean squares (RMS). The linear model was a slightly better fit for the SP-SM soil. However, the differences between fit in the linear and Langmuir models were minor.

Column and batch coefficient comparison

Linear adsorption coefficients obtained from column testing for TNT (0.362 $\pm$ 0.304 L kg^{-1}) were not statistically different ($p < 0.05$) from adsorption coefficients obtained from batch testing (0.143 $\pm$ 0.100 L kg^{-1}). TNT disappearance

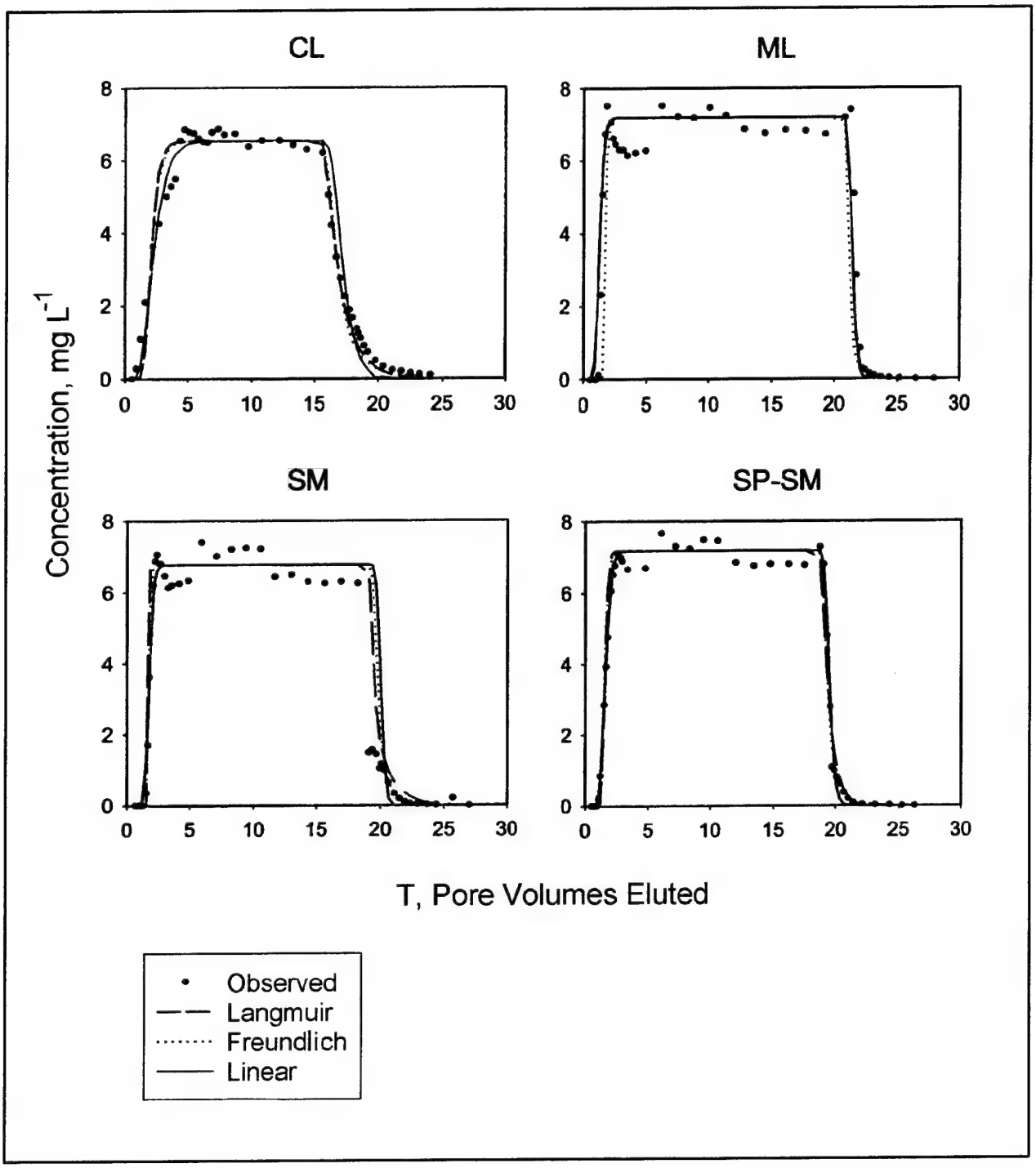

Figure 4. Observed and fitted TNT BTCs for LAAP soils

coefficients did not significantly differ ($p < 0.05$) between batch (0.0008 ± 0.0004 hr^{-1}) and column (0.0016 ± 0.0014 hr^{-1}). These results suggest that results of batch tests, without column tests, are sufficient for generating model input when soil organic carbon is low.

Table 4
TNT Sorption and Transformation Coefficients for LAAP Soils

Model	Soil			
	CL	ML	SM	SP-SM
Linear				
μ^1, sec^{-1}	9×10^{-7}	1×10^{-7}	7×10^{-7}	1×10^{-7}
Kd^2, L kg^{-1}	0.8	0.1	0.3	0.25
RMS[3], mg L^{-1}	0.565	1.036	1.529	0.3054
Freundlich				
μ, sec^{-1}	9×10^{-7}	1×10^{-7}	7×10^{-7}	1×10^{-7}
n^5	0.6	0.05	0.6	0.6
K_f^6, $mg^{(1-n)}$ L^n kg^{-1}	1.5	1.01	0.6	0.4
RMS, mg L^{-1}	0.534	1.657	1.333	0.491
Langmuir				
μ, sec^{-1}	9×10^{-7}	1×10^{-7}	7×10^{-7}	1×10^{-7}
Q^7, mg kg^{-1}	8	3	2	2.15
K_L^8, L mg^{-1}	0.2	0.045	0.75	0.25
RMS, mg L^{-1}	0.515	1.019	1.191	0.530

[1] μ: Transformation coefficient.
[2] K_d: Distribution coefficient.
[3] RMS: Root mean square.
[4] RMS of best fit among linear, Freundlich, and Langmuir isotherms.
[5] n: Constant.
[6] K_f: Freundlich sorption coefficient.
[7] Q: Monolayer sorption capacity.
[8] K_L: Langmuir sorption coefficient.

Site-capacity estimation

The capacity of the LAAP site to attenuate explosives was evaluated using groundwater models in conjunction with the sorption and disappearance rate coefficents. Rapid estimates of site capacity for the LAAP soils from batch equilibrium adsorption data were obtained in two ways. First, the influent concentration for each of the four aquifer soils was substituted into the equation $y = mx + b$ where y is the equilibrium soil concentration (mg kg^{-1}) (site soil capacity), x is the influent concentration (mg L^{-1}), m is the slope (L kg^{-1}), and b is the y-intercept (mg kg^{-1}) (Table 5). Second, isotherm plots of equilibrium soil and solution concentrations (Chapter 3 Appendix Figures 1-3) were used to estimate site capacity. Where a vertical line was drawn from the point on the horizontal axis corresponding to the groundwater concentration contacting a particular soil and the regression line through the data was extrapolated to intersect the equilibrium soil concentration line, then the soil concentration value at the point of intersection with the y axis was the estimated site capacity. This soil concentration value represents the milligrams of contaminant adsorbed per kilogram of soil

Table 5
Site-Capacity Estimation Equation Parameters for LAAP Aquifer Soils

Contaminant	CL Soil		ML Soil		SM Soil		SP-SM Soil	
	Slope, m	*b*[1]	Slope, m	*b*	Slope, m	*b*	Slope, m	*b*
HMX	0.37	-0.04	0.086	-0.01	0.20	-0.04	0.20	-0.07
RDX	0.33	0.15	0.21	0.03	0.33	-0.022	0.33	-0.32
TNB	0.49	0.89	0.16	0.06	0.27	0.15	0.21	0.07
DNB	0.32	-0.005	NSA[2]		NSA		NSA	
TNT	0.27	0.15	0.04	0.08	0.17	-0.10	0.09	0.06
2,4-DNT	0.67	-0.008	0.09	-0.002	NSA		0.28	-0.006
3,5-DNA	0.84	-0.02	0.28	-0.02	0.37	-0.009	0.21	-0.008
Picric acid	0.41	-0.04	NSA		NSA		NSA	

[1] b = y-intercept of equation $y = mx + b$.
[2] NSA = No significant adsorption.

when that soil is in equilibrium with the influent contaminant concentration. For example, the capacity of the soils for RDX given an influent concentration of 1 mg L^{-1} ranges from a low of 0.01 mg kg^{-1} for the SP-SM soil up to 0.48 mg kg^{-1} for the CL soil.

Conclusions

Batch and column results for TNT sorption and disappearance constants showed close agreement for the LAAP site soils. Therefore, batch results adequately described sorption and disappearance rate constants in the LAAP soils. Sorption of explosives compounds by the aquifer soils was limited, with all constitutents showing K_D values below 1 L kg^{-1} for all four soils. The limited range of sorption coefficients values for a particular explosive compound among LAAP soils indicates that a single, average sorption coefficient for each compound in the LAAP soils should adequately describe sorption for numerical modeling. Disappearance rate constants were also low in comparison with those typically observed in surface soils. Most of the disappearance rate coefficients did not differ from, or approached, zero. Use of the disappearance rate coefficents in modeling was complicated by the proximity of the coefficients to zero and the uncertainty that this created about applying results from short-term bench-scale testing to field scale. Use of the disappearance rate coefficents in groundwater models may require adjustment to accurately depict measured groundwater concentrations that reflect field conditions and a longer time frame than is possible with bench-scale batch and column studies. These results suggest that mass transport limitations rather than site capacity restrict transport at LAAP.

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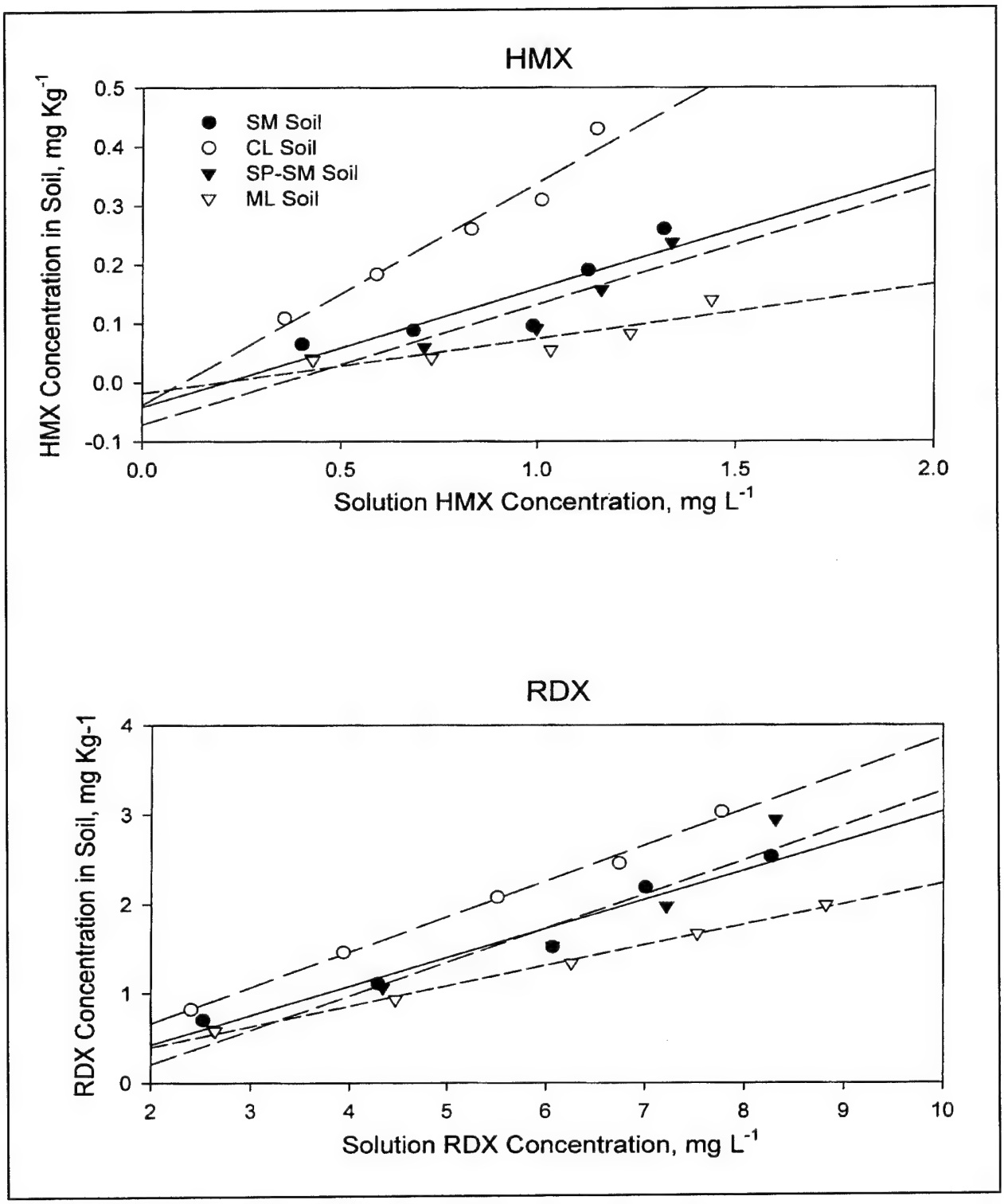
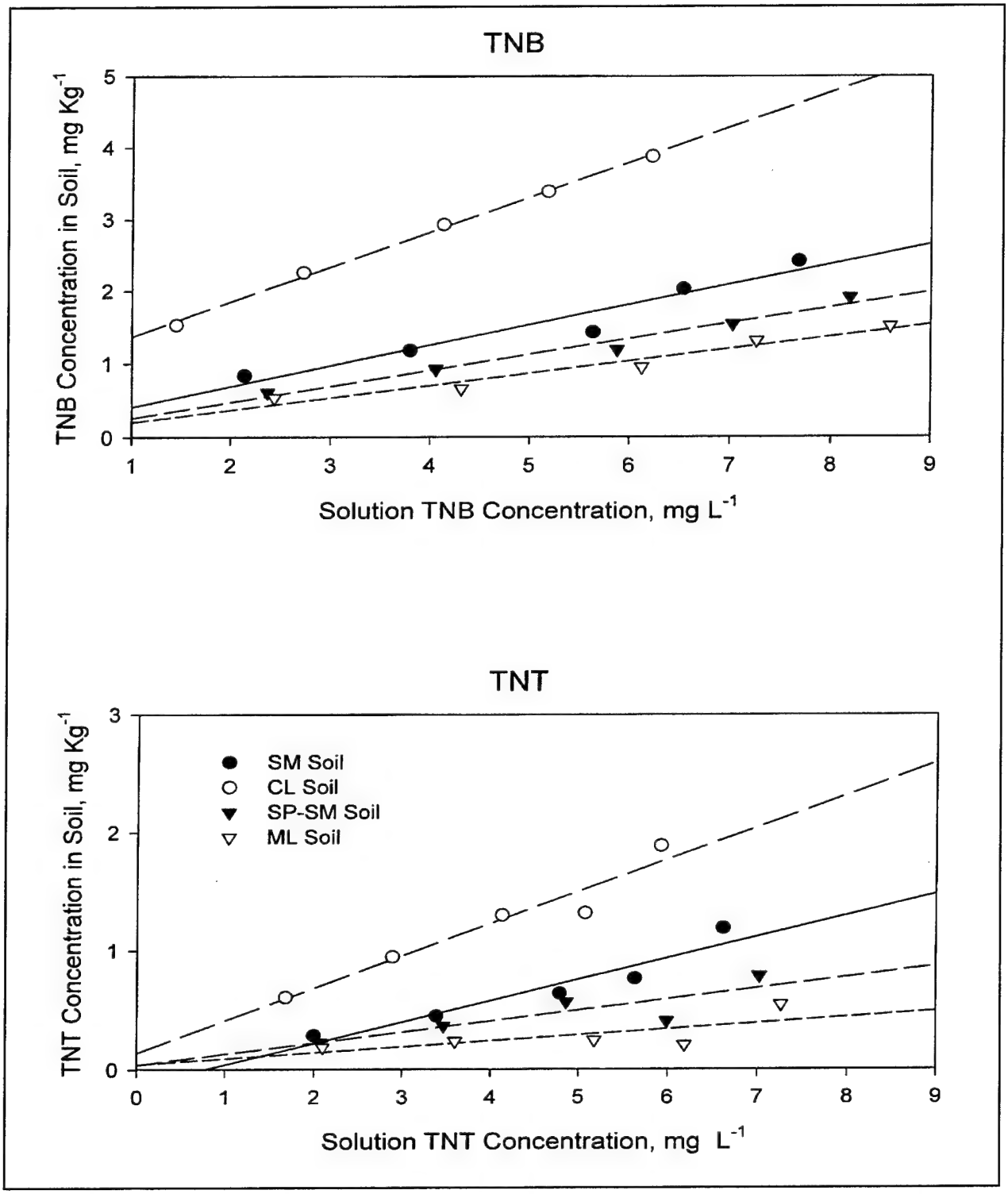
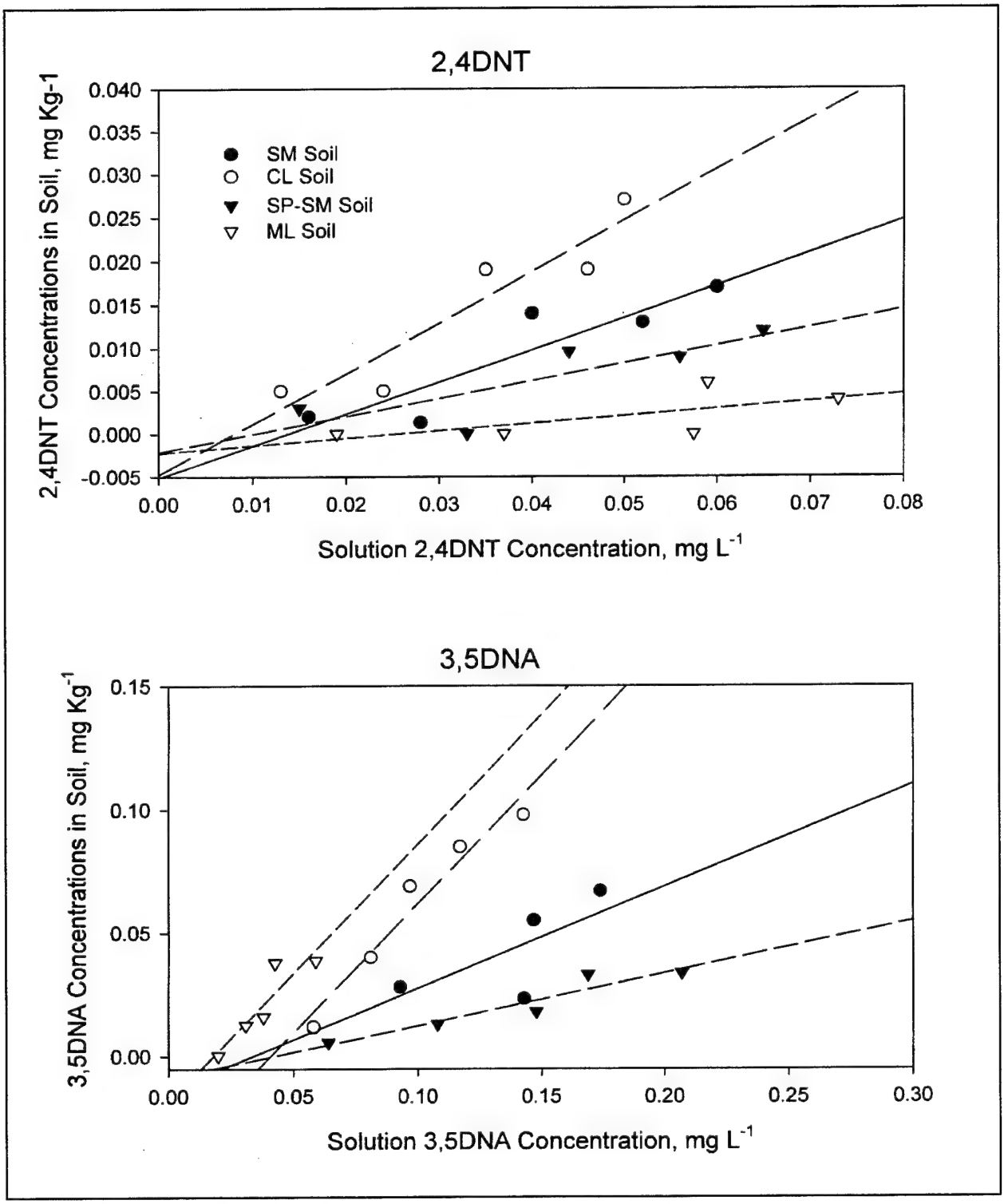
Appendix
Site Capacity



Appendix Figure 1. HMX and RDX adsorption on LAAP aquifer soils and fitted linear adsoprtion model



Appendix Figure 2. TNB and TNT adsorption on LAAP aquifer soils and fitted linear adsorption model



Appendix Figure 3. 2,4DNT and 3,5DNA adsoprtion on LAAP aquifer soils and fitted linear adsorption model

Appendix Table 1
Solution Concentrations (mg L^{-1} (standard error)) in Water from MW85U Exposed to ML Silt from LAAP Aquifer

Time, hr	HMX	RDX	TNB	DNB	TNT	2ADNT	2,4DNT	Picric Acid
0[1]	1.57	9.46	10.1	0.045	7.66	0.30	0.076	0.603
0.5	1.43 (0.02)	8.58 (0.03)	8.75 (0.08)	0.04 (0.004)	7.12 (0.03)	0.26 (0.002)	0.068 (0.003)	0.486 (0.003)
1	1.43 (0.01)	8.81 (0.03)	8.81 (0.05)	0.046 (0.006)	7.15 (0.04)	0.26 (0.002)	0.065 (0.0007)	0.41 (0.006)
6	1.44 (0.003)	8.79 (0.06)	8.72 (0.02)	0.052 (0.0006)	7.07 (0.01)	0.26 (0.002)	0.065 (0.002)	0.41 (0.002)
24	1.45 (0.01)	8.82 (0.05)	8.54 (0.04)	0.041 (0.004)	6.94 (0.03)	0.26 (0.003)	0.062 (0.001)	0.435 (0.025)
48	1.44 (0.01)	8.75 (0.07)	8.35 (0.09)	0.048 (0.006)	6.67 (0.17)	0.25 (0.006)	0.06 (0.002)	0.43 (0.03)
72	1.47 (0.04)	8.70 (0.03)	8.30 (0.04)	0.043 (0.003)	6.70 (0.02)	0.25 (0.002)	0.054 (0.001)	0.43 (0.03)
96	1.42 (0.01)	8.75 (0.05)	8.28 (0.1)	0.037 (0.006)	6.69 (0.07)	0.25 (0.004)	0.057 (0.0006)	0.46 (0.02)
168	1.41 (0.01)	8.74 (0.02)	7.98 (0.09)	0.034 (0.0003)	6.48 (0.05)	0.24 (0.002)	0.05 (0.004)	0.52 (0.027)

[1] Initial values represent single measurement.

Appendix Table 2
Solution Concentrations (mg L^{-1} (standard error)) in Water from MW85U Exposed to SP-SM Sandy Silt from LAAP Aquifer

Time hr	HMX	RDX	TNB	DNB	TNT	2ADNT	2,4DNT	Picric Acid
0[1]	1.57	9.46	10.1	0.045	7.66	0.30	0.076	0.603
0.5	1.37 (0.01)	8.15 (0.04)	8.42 (0.06)	0.031 (0.001)	6.95 (0.03)	0.23 (0.002)	0.062 (0.001)	0.59 (0.07)
1	1.39 (0.03)	8.34 (0.01)	8.42 (0.08)	0.033 (0.0003)	7.01 (0.05)	0.24 (0.002)	0.066 (0.0006)	0.61 (0.015)
6	1.43 (0.003)	8.38 (0.03)	8.42 (0.05)	0.035 (0.002)	7.00 (0.02)	0.24 (0.003)	0.069 (0.003)	0.66 (0.021)
24	1.42 (0.01)	8.39 (0.04)	8.16 (0.11)	0.034 (0.001)	6.82 (0.05)	0.24 (0.004)	0.061 (0.002)	0.615 (0.07)
48	1.41 (0.02)	8.36 (0.11)	8.03 (0.24)	0.031 (0.002)	6.69 (0.11)	0.23 (0.008)	0.057 (0.001)	0.75 (0.01)
72	1.41 (0.02)	8.38 (0.11)	8.00 (0.18)	0.028 (0.001)	6.66 (0.08)	0.23 (0.006)	0.057 (0.003)	0.75 (0.01)
96	1.41 (0.00)	8.38 (0.02)	8.11 (0.07)	0.028 (0.003)	6.65 (0.08)	0.23 (0.001)	0.054 (0.004)	0.73 (0.01)
168	1.37 (0.01)	8.25 (0.03)	7.62 (0.19)	0.025 (0.002)	6.30 (0.11)	0.22 (0.004)	0.05 (0.002)	0.61 (0.11)

[1] Initial values represent single measurement.

Appendix Table 3
Solution Concentrations (mg L^{-1} (standard error)) in Water from MW85U Exposed to SM Silty Sand LAAP Aquifer Soil

Time hr	HMX	RDX	TNB	DNB	TNT	2ADNT	2,4DNT	Picric Acid
0[1]	1.50	9.17	9.65	0.057	7.64	0.281	0.072	0.589
0.5	1.27 (0.01)	8.08 (0.003)	7.77 (0.01)	0.032 (0.001)	6.85 (0.02)	0.217 (0.001)	0.065 (0.003)	0.74 (0.01)
1	1.28 (0.01)	8.05 (0.02)	7.64 (0.09)	0.030 (0.0006)	6.75 (0.06)	0.215 (0.002)	0.064 (0.002)	0.760 (0.004)
6	1.28 (0.02)	8.00 (0.05)	7.55 (0.06)	0.031 (0.001)	6.67 (0.02)	0.211 (0.004)	0.065 (0.003)	0.66 (0.103)
24	1.29 (0.02)	7.96 (0.03)	7.30 (0.10)	0.028 (0.001)	6.52 (0.09)	0.21 (0.004)	0.056 (0.003)	0.780 (0.005)
48	1.28 (0.003)	7.95 (0.05)	7.21 (0.07)	0.026 (0.001)	6.36 (0.06)	0.20 (0.003)	0.052 (0.003)	0.750 (0.01)
72	1.27 (0.01)	7.89 (0.01)	7.01 (0.10)	0.026 (0.002)	6.22 (0.09)	0.20 (0.004)	0.054 (0.005)	0.75 (0.004)
96	1.29 (0.01)	7.91 (0.03)	7.01 (0.09)	0.025 (0.002)	6.19 (0.10)	0.20 (0.005)	0.050 (0.004)	0.671 (0.11)
168	1.28 (0.01)	7.94 (0.05)	7.15 (0.32)	0.023 (0.002)	6.03 (0.05)	0.20 (0.006)	0.046 (0.003)	0.75 (0.02)

[1] Initial values represent single measurement.

Appendix Table 4
Solution Concentrations (mg L^{-1} (standard error)) in Water from MW85U Exposed to CL Lean Clay from LAAP Aquifer

Time hr	HMX	RDX	TNB	DNB	TNT	2ADNT	2,4DNT	Picric Acid
0[1]	1.50	9.17	9.65	0.057	7.64	0.281	0.072	0.589
0.5	1.29 (0.007)	8.02 (0.04)	7.49 (0.09)	0.031 (0.001)	6.62 (0.03)	0.205 (0.003)	0.059 (0.001)	0.774 (0.002)
1	1.26 (0.01)	7.97 (0.04)	7.28 (0.16)	0.030 (0.0009)	6.54 (0.07)	0.198 (0.004)	0.058 (0.001)	0.67 (0.10)
6	1.15 (0.01)	7.66 (0.02)	6.26 (0.13)	0.027 (0.001)	5.99 (0.05)	0.172 (0.002)	0.052 (0.001)	0.72 (0.001)
24	1.17 (0.01)	7.68 (0.04)	5.78 (0.11)	0.026 (0.0003)	5.77 (0.05)	0.171 (0.003)	0.050 (0.001)	0.70 (0.004)
48	1.16 (0.003)	7.51 (0.03)	5.31 (0.04)	0.026 (0.0003)	5.55 (0.01)	0.163 (0.002)	0.047 (0.001)	0.575 (0.09)
72	1.14 (0.02)	7.52 (0.01)	5.05 (0.10)	0.025 (0.0003)	5.43 (0.06)	0.16 (0.002)	0.042 (0.001)	0.44 (0.01)
96	1.13 (0.02)	7.51 (0.02)	4.82 (0.04)	0.025 (0.001)	5.29 (0.04)	0.16 (0.003)	0.043 (0.001)	0.433 (0.006)
168	1.17 (0.003)	7.57 (0.05)	4.61 (0.04)	0.024 (0.0003)	5.20 (0.01)	0.16 (0.001)	0.041 (0.001)	0.43 (0.002)

[1] Initial values represent single measurement.

4 Biomarkers

Rationale

Measurements of in situ contaminant degradation rates are crucial for assessment of cleanup goals. Until now, if chemical data obtained by measurements made directly in the field were not available, evidence for in situ degradation has generally been derived from microcosm data developed under laboratory conditions. However, difficulties in approximating in situ conditions in the laboratory can result in incorrect estimates of in situ contaminant degradation rates. One means for obtaining direct estimates of activity in the field is to relate measurements made on microorganisms under in situ conditions to the performance of these microorganisms measured in laboratory microcosms (ex situ conditions). The relationship between these two measurements can be used to bridge the gap between in situ and laboratory assessments. In situ conditions are reflected in the status of native microbial communities that can be assessed by direct extraction of biomarkers from contaminated soil samples. Biomarkers are biochemical components of microbial cells that indicate ability to degrade contaminants, physiological status, and impacts of exposure to contaminants. Genetic (nucleic acid) and membrane lipid profiles of microbial cells provide information that is particularly relevant to degradation of environmental contaminants. Analysis of gene profiles of microorganisms can reveal the presence or absence of genes necessary for production of enzymes. The presence of these genes is evidence of the catabolic potential of indigenous microflora in situ—i.e., the ability to produce degradative enzymes that are active against the contaminant in the field. Nonspecific degradation and overall community adaptation to pollutants (e.g., clean versus contaminated) are reflected in the amount and type of microorganisms present. Therefore, measurements of microbial biomass and diversity must supplement gene biomarker determinations. Microbial membrane lipids can be measured to assess in situ microbial biomass and community composition.

Nucleic acid and membrane lipid biomarkers can be used to estimate the in situ microbial degradation capability at the site. Degradation potential kinetics for use in predictive groundwater models can be measured by challenging microorganisms from the site with radiolabeled contaminant (radiorespirometry). In this chapter, biomarkers were adopted as a tool for monitoring natural attenuation of explosives by comparison of nucleic acid and membrane lipid biomarkers to radiorespirometry degradation kinetics developed in laboratory microcosms.

Biomarkers were evaluated in soil samples from the LAAP and JAAP explosives-contaminated sites. The relationship between in situ nucleic acid and membrane lipid biomarkers and the contaminant and daughter products present under in situ conditions was determined. This was related to laboratory-derived kinetics to develop more accurate estimates of in situ contaminant degradation rates.

Objectives

The goal of the study was to apply biomarkers as tools to obtain more accurate estimates of in situ contaminant degradation. Specific objectives were as follows:

a. Determine the potential of LAAP and JAAP soils to mineralize explosives.

b. Use genetic biomarkers to provide evidence of in situ explosive catabolic potential based on identification of relevant genes.

c. Use membrane lipid biomarkers to determine the composition and physiological status of in situ microbial communities in relation to contaminant and daughter products in LAAP and JAAP soils.

d. Estimate rates of in situ explosive degradation/transformation by extrapolation from measured rates in laboratory experiments and by correlation of in situ membrane lipid and genetic biomarkers.

Approach

Radiorespirometry (degradation potential)

Radiorespirometry is an evaluation of the rate and extent of microbial degradation under controlled laboratory conditions (Fulthorpe, Rhodes, and Tiedje 1996; Schmidt and Scow 1997). A field sample (soil or groundwater) is incubated with [^{14}C]-labeled acetate, RDX, or TNT (examples for TNT: Bradley and Chapelle 1995; Gunnison et al. 1997). Microorganisms associated with site soils degrade the radiolabeled compound to produce biotransformation and mineralization products. The $^{14}CO_2$ formed from the parent [^{14}C]-labeled compound is trapped and periodically monitored. At the end of the study, the residual radioactivity present in the soil and aqueous portions of the microcosm are assessed. If necessary, individual radiolabeled transformation products can be separated from these media, extracted, identified, and quantified. The rate and extent of product release is evaluated, and the degradation products are identified.

The ability to mineralize substantial amounts of acetate, a readily available carbon source, within a short time frame (2-5 days) is an indicator of the overall health of soil microbial communities. Rapid metabolism that evolves 30 to 60 percent of the added ^{14}C-acetate as $^{14}CO_2$ within this time interval indicates the

presence of a robust microbial population that is not severely impacted by the presence of the contaminants. By contrast, low levels of acetate mineralization suggest that soils are depauperate in microorganisms, lack sufficient nutrients to support high-population levels, or contain xenobiotic compounds at levels toxic to microorganisms. Therefore, radioassays of acetate degradation were included in this study to measure the general health and activity of the indigenous microflora. Production of radiolabeled $^{14}CO_2$ from [^{14}C]-labeled RDX or TNT indicates that microbial communities have the ability to mineralize the respective explosive. The rate of $^{14}CO_2$ evolution can be monitored and a degradation rate determined. Radioespirometry is effective in the detection of biotransformation and mineralization products at low concentrations in the parts-per-billion (ppb) range (Subba Rao and Alexander 1982) and can also be used to monitor the impacts of sorption and desorption on biodegradation of amino compounds (Wszolek and Alexander 1979).

Genetic biomarkers (catabolic genes)

Contaminant biodegradation rates are directly related to the function of degradative enzymes produced by specific microbial genes. If the presence of degradative genes can be linked to actual contaminant destruction (i.e., mineralization in a radiorespirometry study), the microorganisms present in the soil sample are likely to have (a) the catabolic potential to destroy the compound in the field, and (b) the ability to express the enzymes and carry out the degradation process. The number of catabolic genes and levels of gene expression are good indicators of contaminant biodegradative activity in environmental samples (i.e., the greater the number of genes expressed and types of catabolic genes identified, the greater the biodegradation activity). Several investigators have shown a quantitative relationship between levels of a contaminant, degrading gene expression, and degradation of the contaminant in contaminated soils (Fleming, Sanservino, and Saylor 1993; Wikstrom et al. 1996; Stapleton et al. 1998). For this reason, single time-point environmental nucleic acid measurements can be interpreted in terms of in situ biodegradation rates. In like manner, levels of enzymes present in degradative bacteria have been correlated with levels of gene expression (Selenska and Klingmuller 1992; Nazaret et al. 1994; Jeffrey, Nazaret, and Barkay 1996). Detection of genes coding for degradation of explosives in soil samples thus represents the catabolic potential of the native microorganisms. The identification of specific catabolic genes can then be related to contaminant transformation in situ, and environmental nucleic acid measurements can be interpreted in terms of in situ biodegradation rates.

Polymerase chain reaction (PCR) is used to detect the presence of catabolic genes among a complex background of thousands of different genes. Short pieces of DNA 18 to 30 nucleotides long, or "primers," are designed to be identical to small regions within a gene. A specific gene is detected after PCR amplification of primer tagged regions within the gene. These amplified gene fragments are detected by size-separation in polyacrylamide gel electrophoresis. Visible bands (nucleic acid biomarkers) in the gel indicate the presence of the target gene in the

extracted soil sample. A procedure called multiplex PCR provides for simultaneous detection of multiple target genes. With this approach, the presence of two or more different target genes can be identified in a single assay.

PCR primers have been designed to detect genes encoding enzymes for transformation and destruction of TNT according to the pathway shown in Figure 1 (Rieger and Knackmuss 1995) (strains serving as sources of genes shown in Table 1; selected primers shown in Table 2). Examination of all soil samples for all of the enyzmes involved in a pathway is not economically possible; thus certain enzymes key to the degradation pathway are selected. Field soil samples are then examined for genes encoding targeted key enzymes.

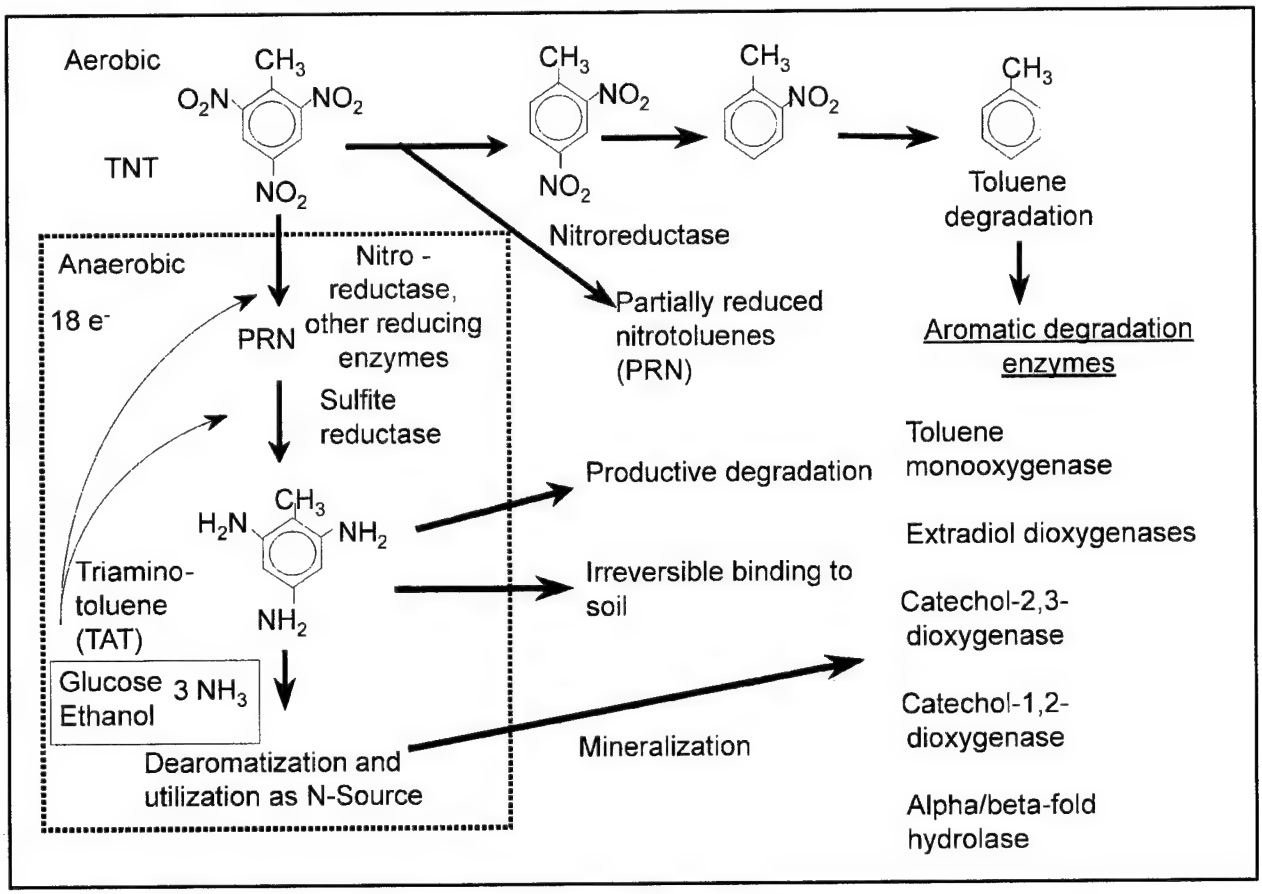


Figure 1. Postulated pathway for degradation of TNT in the environment (adapted from Rieger and Knackmuss 1995) (Enzymes that have been identified for a reaction are indicated)

Lipid biomarkers (biomass and community composition)

Characterization of membrane lipids was used to assess in situ microbial biomass and community composition. Viable biomass can be estimated from the total concentration of ester-linked phospholipid fatty acids (PLFA) present in environmental samples (White et al. 1979). Since intact phospholipids are subject

Table 1
Strains Used

Strain	Phenotype/Genotype	Source
Pseudomonas putida mt-2, pWW0	Extradiol-monooxygenases; toluene and xylene; meta-cleavage pathway (catechol-2,3-oxygenase, C23O gene)	ATCC 33015
Escherichia coli WB-C 49	NAD(P)H flavin nitroreductase (nfsB/nfnB)	Sigma 1994
Desulfovibrio vulgaris subsp. vulgaris	Sulfate reducer, [NiFe] hydrogenase, sulfite reductase (dsrB)	Widdel 1994 (Widdel 1988)
Burkholderia sp. Strain JS150	Toluene/benzene-2-monooxygenase; degradation of benzene, toluene, ethylbenzene, chlorobenzene, 1,4-dichlorobenzene, bromobenzene, 1,4-dibromobenzene, iodobenzene, napthalene, phenol, salicylate, oxidative transformation of nitrotoluenes, (merRP genes)	DSM strain number 8530 (Robertson et al., 1992; Haigler, Pettigrew, and Spain 1992)
Pseudomonas sp. GCC190	PCB degradation (biphenyl dioxygenase gene)	ATCC 53643
Pseudomonas putida F1	Degrades benzene, toluene, toluene-4-monooxygenase (todC1)	ATCC 700007
Burkholderia sp. Strain DNT	2,4-dinitrotoluene mineralization	Spain 1995
Pseudomonas sp. Strain ADP	Atrazine degradation; atrazine chlorohydrolase genes (atzA) and hydroxyatrazine ethylaminohydrolase (atzB)	de Souza, Sadowski, and Wackett 1996
Pseudomonas oleovorans TF4-1L	Epoxidizes terminal olefins (alkB); alkane degradation	ATCC 29347

to rapid catabolism by both endogenous (present within the cell) and exogenous (present in the environment outside the cell) phospholipases (phospholipid-degrading enzymes), their presence is an indicator of the viable biomass (Vestal and White 1989). PLFA are sufficiently distinct to allow for the identification of individual species of bacteria (Guckert et al. 1991). Since different functional groups of microorganisms (such as gram-positive or gram-negative bacteria) utilize different pathways for phospholipid fatty acid biosynthesis, each group has a unique pattern of fatty acids by which that group can be identified. In addition, an interpretation of microbial community composition can be made by utilizing the same knowledge base (Frostegard, Baath, and Tunlid 1993). Community composition may also be inferred from a PLFA profile by relating the specific type of PLFA to different biosynthetic pathways utilized in fatty acid synthesis (Fredrickson et al. 1995) or to specific classes of bacteria that contain a signature PLFA biomarker (Vestal and White 1989). Similarly, sterols have been used to taxonomically relate species of algae, fungi, and protozoa. By determining the sterol content in a sample matrix, insight into the composition of the microeukaryotic populations can be obtained. Results of the signature lipid biomarker analysis are interpreted as described in Ringelberg et al. (1997).

Examples of bacterial species or bacterial groups for which unique signature fatty acid biomarkers have been identified include *Desulfomonile tiedjei,* Type II methylotrophs (Guckert et al. 1991; Ringelberg et al. 1994) and the *Clostridia* (a low G + C gram-positive bacterium). The *Clostridia* has the capacity to reductively deaminate nitroaromatics, resulting in the formation of nitroso-, hydroxylamino-, and aminonitroaromatic compounds (Funk et al. 1993). Sulfate-reducing bacteria, such as *Desulfovibrio* sp., which are also able to reduce sulfite, can be involved in the anaerobic reduction of TNT (Figure 1). As a phenotypic

Table 2
Primers and Targets Used in Multiplex PCR Analysis of LAAP and JAAP Soils

Primer	Product Size	Sequence	Target[1]	Strain Standard	Soil	Ref[2]
merR merP	1002	GGGAGATCTAAAGCACGCTAAGGCRTA GGGGAATTCTTGACWGTGATCGGGCA	Tn501 mercury resistance genes operon	*Burkholderia* JS150	LAAP JAAP	a
todC1F todC1R	924	GCGAGATAGAAGCGGCTCTTG GTATTGATACCTGGGAGGAAG	Catabolic, aromatic, extradiol dioxygenase, iron-sulfur subunit, toluene dioxygenase	*Pseudomonas putida* F1 todC1	JAAP LAAP	b
tmoAF tmoAR	900	GCTATGTTACCGAAGAGCAGC GGAATAGATCCCAGTACCAGG	Catabolic, toluene-4-monooxygenase, tmoA	*Pseudomonas mendocina* KR1	LAAP JAAP	b
alkB-f alkB-r	869	TGGCCGGCTACTCCGATGATCGGAATCTGG CGCGTGGTGATCCGAGTGCCCTGAAGGTG	Catabolic, alkane, alkane hydroxylase (alkB)	*Pseudomonas oleovorans* ATCC 29347	LAAP JAAP	c
amoAF amoAR	665	AGAATCCTGAAAGCGGC GATACGAACGCAGAGAG	Nitrogen, ammonia monooxygenase	*Nitrosomonas europae*	LAAP JAAP	d
24DNT702F 24DNT1211R	535	TAWGCTTCCACCCGAAGGCYCG TCGCYGAWTGCCGATTTGCYAACGA	Catabolic, aromatic, extradiol dioxygenase, iron-sulfur subunit, 2,4-dinitrotoluene reductase (dntAc), nitrotoluene reductase (ntdAc)	*Burkholderia* sp. DNT	LAAP	d
27fp 519r	530	AGAGTTTGATCMTGGCAG GWATTACCGCGGCKCGTG	Structural, translation, 16s rDNA of bacteria	*P. oleovorans* ATCC 29347	JAAP	e
hyd1F hyd5R	430 438, 444	CGCGACGCCCAGCACTTCACCCAGCGC GCAGGGCTTCCAGGTAGTGGGCGGTGGCGATGAGGT	*Desulfovibrio* sp. (NiFe)-hydrogenase gene	*Desulfovibrio vulgaris* sp. *vulgaris*	LAAP	f
Sigma54-f Sigma54-R	450	TCAAGCTTACRTTNCCNGGVMA GAGAATTCGARYNTTYGGNCAYG	Regulatory activator, sigma 54-dependent activators	*P. putida* pWWO	LAAP	g
dsrB2002f/ dsrB2338r	363	CAGGGCTGGRTNMACTGCCACAC GTGTAGCAGTTNCCRCAGTACATGCA	Dissimilatory sulfite reductase (dsrB) targets Fe-S ferredoxin binding site	*Dsulfovibrio vulgaris*	JAAP	d
2NT-F 2NT-R	314	TTTGTGTGCGGYTACCACGGNTGGCA TCTCACCTACAAAGTTTTCCGACAAARSCTTCCAGTT	Catabolic, aromatic, extradiol dioxygenase, iron-sulfur subunit	*Burkholderia* sp. DNT	LAAP JAAP	d
nreduct1F/ nreduct1R	381-430	GCACCAAYTCCCAGCCNTGGCA GCGGCGTCRAAACCTTCNATKGGKAC	Nitro-reducing, NAD(P)H flavin reductase	*Escherichia coli* nfsB/nfnB, *Salmonella typhimurium*	LAAP JAAP	d
bph-f bph-r2	295	TGCAGCTACCACGGCTGGGCCTA GCNGCRAAYTTCCARTTRCANGG	Catabolic, aromatic, extradiol dioxygenase, iron-sulfur subunit, biphenyl dioxygenase	*Pseudomonas* sp. GCC190	LAAP JAAP	d
c203f c2303r	202	CAAGGCCCACGACGTGGCNTT CGGTTACCGGACGGGTCGAAGAAGT	Catabolic, aromatic, extradiol oxygenase, meta cleavage, catechol-2,3-dioxygenase (xylE type)	*P. putida* pWWO	LAAP JAAP	d
azb883F atzb1057R	174	TCGATGGCATAATATCTGCGTTGCG GATCCGGGCCAAGGCCAATAATCAC	Catabolic, atrazine degradation *Pseudomonas* sp. strain ADP atzB and atzA	*P.* sp. strain ADP	LAAP JAAP	d

[1] Unless otherwise indicated, all genes are functional.
[2] References: a,Osborn et al. 1993; b, Zylstra and Gibson, 1989; c, Kok et al., 1989; d, This report; e, Lane, 1991; f, Wawer and Muyzer, 1995; g, Kaufman and Nixon, 1996.

expression, certain membrane lipids (i.e., PLFA) change in response to fluctuations in environmental conditions. Insight into the physiological status of the in situ microflora can be obtained by quantifying these changes. For example, environmental stress exerted by toxicant exposure and starvation can be evaluated for gram-negative bacterial populations by measuring accumulations of *trans* fatty acids (Kieft et al. 1994). Specific microorganisms and their physiological status can be quantified and monitored using signature fatty acids in the environment upgradient and within a zone of contamination before and after the initiation of remedial actions.

To quantitatively recover membrane lipids, total lipid extracts are produced from soil samples using a mixture of organic solvents (Bligh and Dyer 1959). Typically, the total lipid extract is fractionated on a silica gel column (Guckert et al. 1985) and then derivatized and analyzed with gas chromatography/mass spectrometry (GC/MS) using single quadrapole or ion trap mass spectrometers. An extraction apparatus (such as the accelerated solvent system) can be coupled with simple class fractionation and a singe-step derivatization process to automate the analysis. Through this process, a rapid (~4 hr) onsite analysis of the native microbial biomass and community composition can be achieved.

Materials and Methods

Soil samples

Selected soil cores from LAAP and JAAP CPT sampling events were sieved using a sterile No. 4 sieve (screen mesh size 4.75 mm) fitted with a sterile catch pan. All operations were conducted in a GermFree fully vented biofume hood (GermFree Laboratories, Inc., Miami, FL). The soil sample was continuously smeared over the surface of the sieve screen using a sterile spatula until all material small enough to pass the screen was forced through. The catch pan and sieve were separated, and the sieve was placed into a basin of soapy water outside the hood for decontamination and resterilization.

The sieved material was thoroughly mixed to ensure homogenization, and subsamples were allocated for each of the following tasks:

Task	Wet Weight, g
Nucleic acid analyses	84
Membrane lipid analyses	84
Radiorespirometry	125
Explosives analysis	10
Stable isotope analyses	20
Particle size analyses	67
Other geochemical analyses	110
Total	500

All subsamples were stored in Whirl-Pak bags (Weatherby/NASCO, Fort Atkinson, WI). All samples except those slated for radiorespirometry were immediately frozen at -80 °C until used. Geochemistry and particle size analyses were conducted according to the methods given in Chapter 2, Groundwater Monitoring and Cone Penetrometry.

Radiorespirometry of soil samples

Following sieving, homogenization, and mixing, 1.0 g subsamples of each soil were placed into numbered, predried, and tared aluminum weighing dishes and dried overnight at 105 °C. Dried samples were allowed to come to room temperature in a desiccator and then reweighed. The percent moisture was determined by dividing the dried weight by the initial moist weight. Some of the same soils were reserved for nutrient analysis and the examination of nucleic acid and lipid biomarkers.

Thirteen 250-mL biometer flasks (Bellco Glass, Inc., Vineland, NJ) were prepared for each soil sample by washing thoroughly, rinsing with concentrated hydrochloric acid, and rinsing again with distilled water to remove the acid. Each biometer was then cleaned in a steam-cleaning dishwasher and autoclaved prior to use. Two milliliters of acetone was added to each flask, swirled, and allowed to sit for 5 min. One milliliter of the acetone was counted by liquid scintillation (LS). This ensured that all traces of radioactivity from previous studies had been removed.

A 30-percent soil/water slurry was produced by mixing 9 g of sieved and homogenized soil with 21 mL of sterile RO water to yield a total effective volume of 30 mL in each biometer. The sterile controls were derived by autoclaving biometers containing the 30-percent soil slurries twice at 121 °C for 15 min each time.

Parallel testing of acetate, RDX, and TNT was conducted. In each case, one autoclaved and three unautoclaved biometers were injected with radiolabeled compounds. Each flask received 0.2 μCi doses of the radioisotopes shown below.

Test Constituent	Initial Concentration of Compound		Specific Activity (mCi) ($mmol^{-1}$) and Source of Material	Sample Schedule
	mMole	mg L^{-1}		
Acetate	2.2×10^{-1}	13.2	110, ICN Biomedical, Inc., Irvine, CA	1, 3, 5, 8, 12 hr, 1, 2, 3, 4, 5 days
RDX	9.6×10^{-2}	21.3	48.0, New England Nuclear Research Products, Boston, MA	1, 2, 3, 4, 5, 6, 7, 10, 14, 17, 21, 28 days
TNT	8.2×10^{-3}	1.86	4.12, New England Nuclear Research Products, Boston, MA	1, 2, 3, 4, 5, 6, 7, 10, 14, 17, 21, 28 days

Flasks were swirled on a gyrorotary shaker for approximately 5 min. Two milliliters of fresh 1N KOH was added to each sidearm, and the flasks were sealed with sterilized stoppers. The flasks were incubated on the shaker at 100 rpm and room temperature (23.3 ± 3.2 °C). At each sampling time, 2.0 mL of KOH from each sidearm was removed and replaced, and 1.0 mL was counted. Identical quantities of radiolabeled spikes were pipetted into triplicate scintillation vials and counted to serve as time zero values.

At the end of the incubation, the KOH was removed, 2.0 mL of fresh KOH was added to the sidearm, and approximately five drops of concentrated H_3PO_4 were added to the main well of each flask. The flask was resealed and shaken for 24 hr at room temperature. The following day, this KOH was removed, and 1.0 mL was counted. In addition, a 1.0-mL sample was taken from the main well of each biometer with shaking. Portions of the liquid phase from each biometer flask were separated from the solid phase by centrifugation at an RCF of 676 for 20 min. The radioactivity in the liquid phase was counted. The solid pellet was dried at 105 °C, combusted (Packard Solid Sample Oxidizer, Packard Instruments, Inc., Downers Grove, IL), and the $^{14}CO_2$ quantified by LS.

Following completion of the radiorespirometry, $^{14}CO_2$ counts from each replicate run on the same soil with the same isotope were summed cumulatively for the time incubated, divided by the radioactivity initially added to each flask (0.2 μCi or 444,000 dpm), and then averaged for each time interval. The data were plotted graphically. Linear rate equations were developed for each curve using the total radioactivity accumulated over the incubation period (expressed as a percent of the activity added) divided by the incubation period in days. In addition, average values of $^{14}CO_2$ counts for the solid and liquid phase of each set of biometer flasks were derived after correction for total volume of material present in each biometer, expressed as a percentage of the total initially present. The average values for liquid and solid phases were summed with the total cumulative $^{14}CO_2$ values to yield mass balances for each soil-isotope combination. Details for radiorespirometry work are also presented in Gunnison et al. (1996, 1997) and in Pennington et al. (1998).

Nucleic acid biomarkers

Genetic standards and target primers. Strains and associated phenotype/genotypes for microorganisms used in this study are listed in Table 1. Polymerase Chain Reaction (PCR) primers were selected for size discrimination and annealing temperatures of approximately 60 °C (Table 2). PCR primers were synthesized on an Expedite DNA/RNA synthesizer (PerSeptive Biosystems, Framingham, MA). Primers were designed using backtranslated multiple sequence alignments for functional enzymes of interest (references available at the following internet addresses: http://www.mcs.anl.gov/home/compbio/PUMA/Production/puma_graphics.html and http://www.cme.msu.edu//WIT/). Multiple alignments were created and analyzed using the Genetics Computer Group suite of software

(Genetics Computer Group 1994) in addition to Primer Premier (Biosoft International, Palo Alto, CA).

DNA isolation from LAAP soil. Three methods for DNA extraction from low biomass samples were examined: a microwave lysis with commercial reagents (GeneReleaser, Bioventures, Murfreesboro, TN), FastDNA Soil Kit (Bio 101, Vista, CA) and a minibead beater system (FastPrep Instrument FP 120, BIO 101, Vista, CA), and a hot sodium dodecyl sulfate (SDS) lysis (Zhou, Bruns, and Tiedje 1996, summarized in Chapter 4 Appendix). Of the three, the hot SDS lysis method provided consistent yields of high molecular weight DNA suitable for analysis. Three separate 10-g subsamples from each core were processed using the method of Zhou, Bruns, and Tiedje (1996) with the following modifications: sediment samples were incubated for 2 hr at 60 °C (instead of 65 °C) in a rotary hybridization oven (Hybaid Instruments, Holbrook, NY). DNA was precipitated overnight (instead of for 1 hr) at room temperature from the extraction buffer using 0.7 (instead of 0.6) volumes of isopropanol and 10 mg of mussel glycogen (in place of 2 mL of 7.3M ammonium acetate) per milliliter (Five prime-Three prime, Boulder, CO). DNA pellets were resuspended into 20-30 μL 1 mM Tris, 10 mM EDTA, pH 8 (instead of being washed with 70-percent ethanol, air dried, and resuspended in 20 µL distilled water). DNA was purified and size selected as described in Young et al. (1993) by electrophoresis through a 0.7-percent low melting agarose (Promega, Madison, WI) containing 0.2-percent polyvinylpyrrolidone (average molecular weight of approximately 29,000, Aldrich, Milwaukee, WI) and Tris-Acetate-Ethylenediaminetetraacetic Acid (TAE) electrophoresis buffer (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA, pH 8.3). Gels were run 18 hr at 1 V per centimeter between electrodes. DNA was visualized by staining in 100 mL H_2O with a 1/10,000 dilution of SYBR Green Stain (Molecular Probes, Beaverton, OR) and imaging with an Ambis digital camera system (Scanalytics, Inc., San Diego, CA). DNA yields and molecular weights were estimated by comparison with molecular size standards of known concentrations (HindIII-EcoRI digested Lambda DNA, Gibco-BRL, Gaithersburg, MD) and ONE-Dscan (Scanalytics), a one-dimensional gel analysis package. DNA bands were excised from agarose-polyvinyl pyrrolidone gels and stored at -20 °C. In samples where no DNA band was visible, gel areas in the size range of 25 thousand base pairs (Kb) were excised and stored at -20 °C for further analysis.

Polymerase chain reaction (PCR) conditions for LAAP soils. PCR conditions depended on the biomass available as determined by nucleic acid or PLFA analysis. For the LAAP study where minimal amounts of biomass were available, the PCR system consisted of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 5 percent dimethylsulfoxide, 500 mg bovine serum albumin per milliliter, 200 femtomoles (fM) of each deoxynucleoside triphosphate, 8 picomoles of each primer, 0.8 units of AmpliTaq Gold DNA polymerase (Perkin Elmer Applied Biosystems, Foster City, CA), and 9 μL sediment DNA in low melting point agarose for a final reaction volume of 20 µL. PCR reactions were amplified in 200-µL thin-walled tubes using a PTC-200 thermal cycler (M. J. Research, Watertown,

MA). The full thermal profile sequence for amplification is given in the appendix to this chapter.

DNA isolation from JAAP soils. Total DNA was isolated using FastDNA Soil Kit (Bio 101, Vista, CA) and a mini beadbeater system (FastPrep Instrument FP 120, BIO 101, (BIO 101, Vista, CA) according to manufacturer's recommendations. Total DNA was isolated from each core sample in three replicated 0.5-g soil subsamples. DNA was eluted into 100 μL of 10mM Tris-thylenediaminetetraacetic Acid (TE) Buffer and stored at -20 °C until analyzed.

Multiplex PCR assay for detection of genes implicated in explosives degradation for JAAP soils. For the JAAP study, where higher levels of biomass were available, the above procedure was modified as follows. PCR primers were selected to allow discrimination of PCR products by size (Table 2). Twelve primers were used simultaneously. A standard gene target mix was created from the nine strains, using 500 pg of total DNA from each strain (Table 1). Assay conditions were developed using the standard mix (Table 1). PCRs were composed of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 5 percent dimethylsulfoxide, 500 mg of bovine serum albumin/L, 250 μM of each deoxynucleoside triphosphate, 8 picomoles of each primer, 0.8 U of AmpliTaq Gold DNA Polymerase (Perkin Elmer Applied Biosystems, Foster City, CA) and 1 μL soil for a final reaction volume of 20 μL. PCRs were amplified in 200-μL thin-walled tubes using a PTC-200 thermal cycler (MJ Research, Watertown, MA). The thermal sequence for this amplification is given in the appendix to this chapter.

Extraction, visualization, sizing, and scoring of PCR products for LAAP and JAAP soils. When appropriate, PCR products were extracted from low-melting agarose by centrifugation (14,000 RCF for 20 min) through Micropure 0.45-μm separators (Amicon, Inc., Beverly, MA) at 10 °C for 5 min. PCR products were separated and analyzed on 20- by 20-cm, 7-percent Long Ranger Hydrolink vertical gels (FMC BioProducts, Rockland, ME). DNA was visualized by staining in SYBER Green I Stain (Molecular Probes, Beaverton, OR) and images captured with an Ambis digital camera system (Scanalytics, Inc., San Diego, CA). DNA sizes were estimated by comparison with molecular size standards of known concentrations (123 bp ladder, Sigma, St. Louis, MO) and ONE-Dscan (Scanalytics, Inc.), a one-dimensional gel analysis package. Multiplex PCR products were identified by amplification of multiplex reaction products using individual primer sets.

Total DNA was extracted from 17 LAAP and 3 JAAP core samples and analyzed by multiplex PCR. Triplicate subsamples were extracted from each core to account for sample heterogeneity (typical multiplex PCR output is shown in Figure 2). Core subsamples were analyzed for the presence and abundance of target catabolic genes by dilution to extinction. Dilutions of total DNA from a subsample were 1/1, 1/10, 1/100, and 1/1000. Multiplex reactions were scored for the presence or absence of bands. The presence of PCR products was

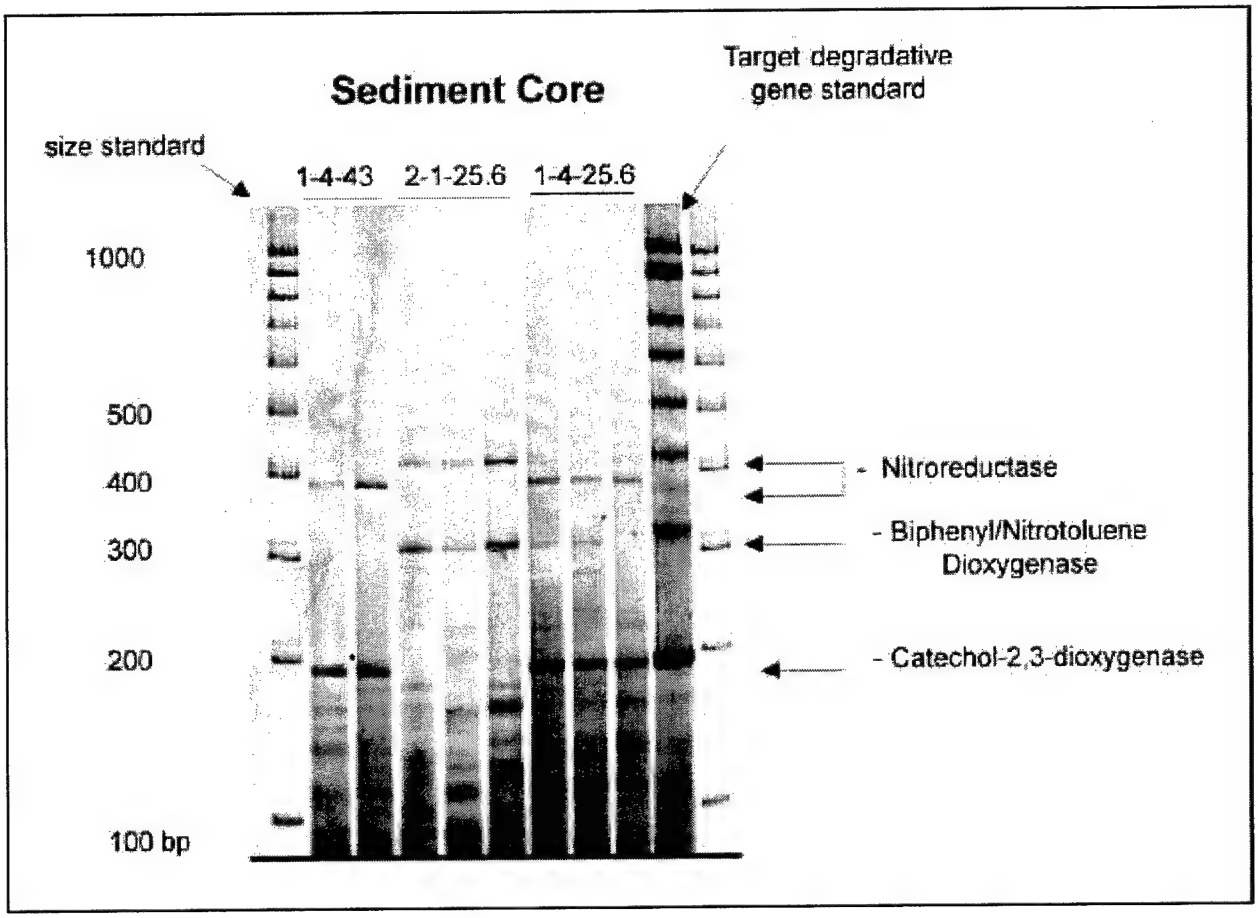


Figure 2. Multiplex analysis of LAAP sediment cores

weighted by the dilution factor, e.g., a band present at a 1/100 dilution would have to be present in at least 2.00×10^4 copies per gram of soil. Therefore, the band would be given a weight of 100. A sample was considered to have a band present if the band was observed at least twice in analysis of three replicate subsamples.

Membrane lipid biomarkers

Lipid extraction. Approximately 60 g of each homogenized soil was placed into a 200-mL centrifuge bottle to which was added dichloromethane-methanol-phosphate buffer (DCM:MeOH:PO_4 at 1:2:0.8, v/v/v) for a total volume of 142.5 mL, excluding any volume occupied by the soil. The material was treated for 2 min in an ultrasonic bath and allowed to extract for approximately 3 hr. The material was centrifuged at 676 RCF for 20 min, and the supernatant was decanted into a 250-mL separatory funnel. The soil was washed with an additional 37.5 mL of DCM, centrifuged, and combined with the initial extractant in the separatory funnel. A two-phase system was established in the funnel by the addition of 37.5 mL of water. After thorough mixing, phases were allowed to

separate overnight (approximately 18 hr). The organic phase was recovered and taken to dryness using rotary evaporation.

Lipid fractionation/preparation. Total lipid from the soil source was fractionated into specific classes using 100 mg of silicic acid packed into a 5-mL volume column containing a glass wool plug. The total lipid was loaded onto the column with 3 × 100-μL washes of DCM. The following classes of compounds were eluted with 5.0-mL washes of DCM, acetone, and MeOH, sequentially:

Step	Elution	Class of Compounds Obtained
1	Silicic acid – DCM	Neutral lipids
2	Silicic acid – Acetone	Glycolipids
3	Silicic acid – Methanol	Polar lipids
	Trans-Esterification Step	
4	Polar-lipids (methanol fraction)	Phospholipid fatty acid methyl esters (PLFAME)

For trans-esterification, the MeOH elutant was evaporated to dryness under nitrogen, dissolved in 1.0 mL of DCM:MeOH (1:1, v:v) to which 1.0 mL of 0.2M methanolic KOH was added. The mixture was heated at 40 °C for 30 min to form the fatty acid methyl esters of the recovered phospholipids (PLFAME). Upon cooling, the PLFAME were recovered in 2 mL of hexane:DCM (4:1, v:v), after additions of 0.2 mL of 1N glacial acetic acid and 2 mL of H_2O.

Instrumental analyses. The PLFAME were further separated and quantified by capillary GC and GC/MS using a nonpolar 60-m cross methyl silica capillary column (32-mm ID, 0.1 μL (film thickness)). The GC was programmed from 80 °C following a 2-min hold, to 150 °C at 10°/min, then to 282 °C at 3°/min and held at 282 °C for 5 min. Detection was by flame ionization detector (FID) and quantification by incorporation of an internal standard (nonadecanoic acid 19:0 at 50 pmol/FL). Detection limit of this system was 5 pg C/sec.

PLFA recovery efficiency and background. Extraction efficiency was determined by performing a spiked recovery experiment. Two bacterial isolates from the WES culture collection, #4005 (*Sphingomonas* sp.) and #21908 (*Arthrobacter* sp.), were grown in nutrient broth, counted, and used to spike pre-extracted soil at cell densities of 10^6, 10^5, and 10^3 cells per milliliter (Table 3). In all cases, the percent recovery was high, although for concentrations of cells at 10^3 mL^{-1}, the test precision was very low.

Table 3
Recovery of Ester-Linked Phospholipid Fatty Acids (PLFA) from Sediment Spiked with Two Bacterial Isolates (n = 2)

Isolate	Cells/mL	pmol PLFA/mL		Percent Recovery
		Cell Suspension	Sediment	
No. 4005 *Sphingomonas* sp.	1.00×10^6	17,632 ± 3,180	51,147 ± 895	>100
	1.00×10^5	1,604 ± 213	4,266 ± 333	>100
	1.00×10^3	111 ± 158	224 ± 209	>100
No. 21908 *Arthrobacter* sp.	1.00×10^6	15,159 ± 767	29,304 ± 1,201	>100
	1.00×10^5	1,581 ± 307	1,733 ± 1,005	>100
	1.00×10^3	93 ± 51	48	52

Data handling and analysis

Total mineralization and individual mineralization rates for each soil-isotope combination were evaluated with reference to nucleic acid biomarkers, membrane lipid biomarkers, explosive and explosive transformation products, and various other geochemical parameters. These correlations were performed using the Spearman Rank Order Correlation or the Pearson Product-Moment Correlation in the SigmaStat Statistical Software Package (McClave and Dietrick 1982; Federle et al. 1986; Jandel Corporation 1995). Regression equations fit to the mineralization kinetics were obtained with the use of simple linear regression, polynomial regression, and nonlinear regression analyses in the same statistical package.

Microbial communities were defined in terms of the contaminants present using multivariate statistical analysis. Principal components analysis and hierarchical cluster analysis were performed on arcsin-transformed percentages of PLFAME concentrations and combinations of genetic markers using the Statistica Statistical Software Package (Statsoft, Inc., Tulsa, OK). Ward's hierarchical cluster analysis method (Ward 1963) was selected because (a) the algorithm is based on variance and (b) the method attempts to minimize within group variance while maximizing between group variance. The bias that biomass may have exerted on the results was decreased by using only samples having a viable biomass of 2.0 pmole PLFA/g dry weight or greater for the analysis.

Nucleic acids and lipid biomarkers were analyzed separately with a nonparametric correlation analysis (Spearman Rank Order, Steele and Torrie 1980) and were combined for a multiple regression analysis. Multiplex PCR results scored for presence or absence of a target gene (unweighted) and weighted values of the results were evaluated individually. For the multiple linear regression analyses, a linear relationship between the dependent and independent variables was assumed and the residuals (predicted minus observed) were assumed to be

normally distributed. Unless otherwise stated, all correlation coefficients were evaluated at the $p < 0.05$ level of significance.

Results for Louisiana Army Ammunition Plant (LAAP)

Radiorespirometry

Mineralization of acetate and explosives. Acetate mineralization in subsurface soil was weak (Table 4) and heterogeneously distributed (Figure 3, Table 4). A value of at least 30-percent mineralization in 5 days would suggest robust microbial populations.

Microbial communities resident in the subsurface exhibited limited potential for mineralization of RDX (Table 4, Figure 3). The distribution pattern for ^{14}C-RDX mineralization activity with depth was completely different from that for acetate (Figure 3). The $^{14}CO_2$ released from radiolabeled RDX at 8.3- and 17.4-m depths at NA 6-2 were bound as carbonates that were primarily released upon acidification (PROA) (Table 4). Moderate levels of RDX mineralization occurred over 28 days, but variability was sometimes high (Figure 3). The rates for RDX mineralization were moderate in comparison to those obtained for TNT (Table 4). TNT mineralization occurred in the surface samples at levels of up to 6 percent above the impurity level (Figure 4). All TNT mineralization rates for subsurface soils were small (≤0.121 percent/day) (Table 4).

Mass balances for acetate and explosives mineralization. Recoveries of ^{14}C from ^{14}C-acetate introduced into subsurface soil varied widely (range from 26 to 98 percent, average of 58.1 ± 4.21 percent) (Figure 5, upper panel). Standard errors for the total mass balances were often large. Mineralization to $^{14}CO_2$ was limited (see above). Most of the radioactivity was found in the aqueous phase with little or no radioactivity accumulating in the solid phase.

Percent recoveries from the subsurface soils receiving radiolabeled RDX (Figure 5, middle panel) were good (average of 67.8 ± 0.544 percent). Most of the radiolabel remained in the aqueous phase; only very small amounts were recovered from the solid phase. Mineralization to $^{14}CO_2$ was limited.

Mass balances for [^{14}C]-TNT averaged 75.3 ± 0.717 percent (Figure 5, bottom panel). However, compared with acetate or RDX, more TNT was bound to the solid phase. Nonetheless, most of the radioactivity was associated with the aqueous phase, and mineralization to $^{14}CO_2$ was limited.

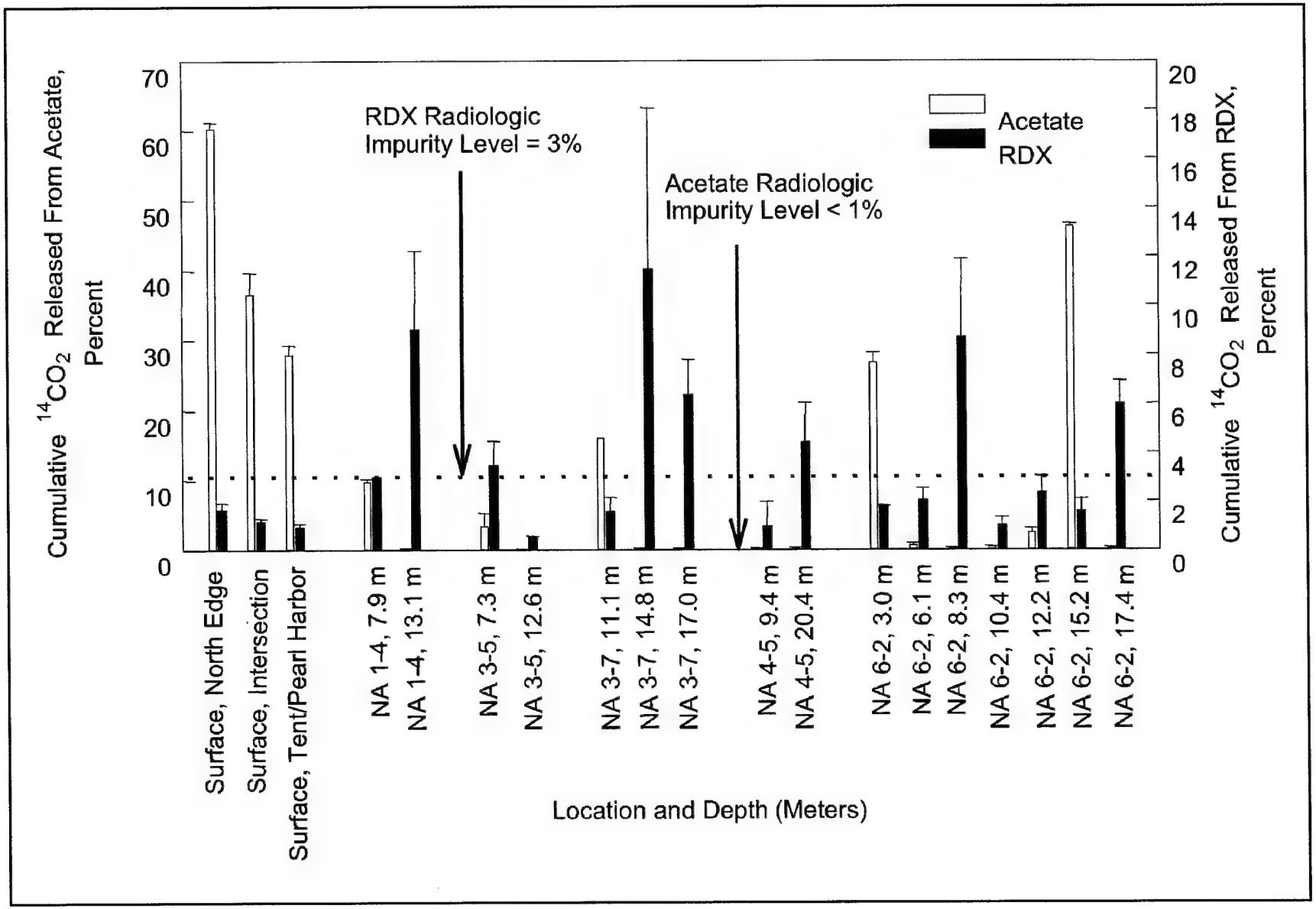


Figure 3. Comparison of total mineralization values for acetate and RDX obtained from LAAP surface and subsurface soils

Table 4
Mineralization Rates from Radioassay Studies on LAAP Soils

Sample Site	Sample Location	Depth m	Acetate Rate %/day[1]	RDX Rate %/day[2]	TNT Rate %/day[2]
Surface	1 (Intersection)	Surface	11.5 ± 0.0	ND[3]	0.063 ± 0.001
	2 (North Edge)	Surface	6.72 ± 0.08	ND	0.064 ± 0.006
	3 (Southwest)	Surface	4.98 ± 0.08	ND	0.219 ± 0.005
Subsurface	1-4	7.9	1.36 ± 0.10	ND	ND
	1-4	13.1	ND	0.214 ± 0.000	ND
	3-5	7.3	ND	0.181 ± 0.012	0.057 PROA[4] ± 0.000
	3-5	12.5	ND	ND	0.032 PROA ± 0.060
	3-7	10.7	2.60 ± 0.836	0.150 ± 0.069	ND
	3-7	14.9	ND	ND	ND
	3-7	17.1	ND	0.121 ± 0.106	0.037 PROA ± 0.094
	4-5	9.4	ND	ND	0.104 PROA ± 0.033
	4-5	20.4	ND	0.051 ± 0.007	0.046 PROA ± 0.039
	6-2	3.0	4.76 ± 0.210	ND	ND
	6-2	6.1	ND	ND	ND
	6-2	8.3	ND	0.204 PROA ± 0.088	0.071 PROA ± 0.064
	6-2	10.4	ND	ND	0.121 PROA ± 0.000
	6-2	12.2	ND	ND	0.121 PROA ± 0.009
	6-2	15.2	8.66 ± 0.000	ND	ND
	6-2	17.4	ND	0.107 PROA ± 0.056	ND

[1] Average acetate rate determined over 5 days ± standard error of the mean.

[2] Average RDX and TNT rates determined over 28 days ± standard error of the mean.

[3] ND indicates that the total level of mineralization did not exceed 3 percent over the 5- (acetate) or 28-day (RDX and TNT) incubation period.

[4] PROA = Primarily released on acidification—most of the $^{14}CO_2$ release occurred on acidification, indicating that the carbon dioxide had previously been bound up as carbonates. All tests were run at 23.3 ± 3.2 °C.

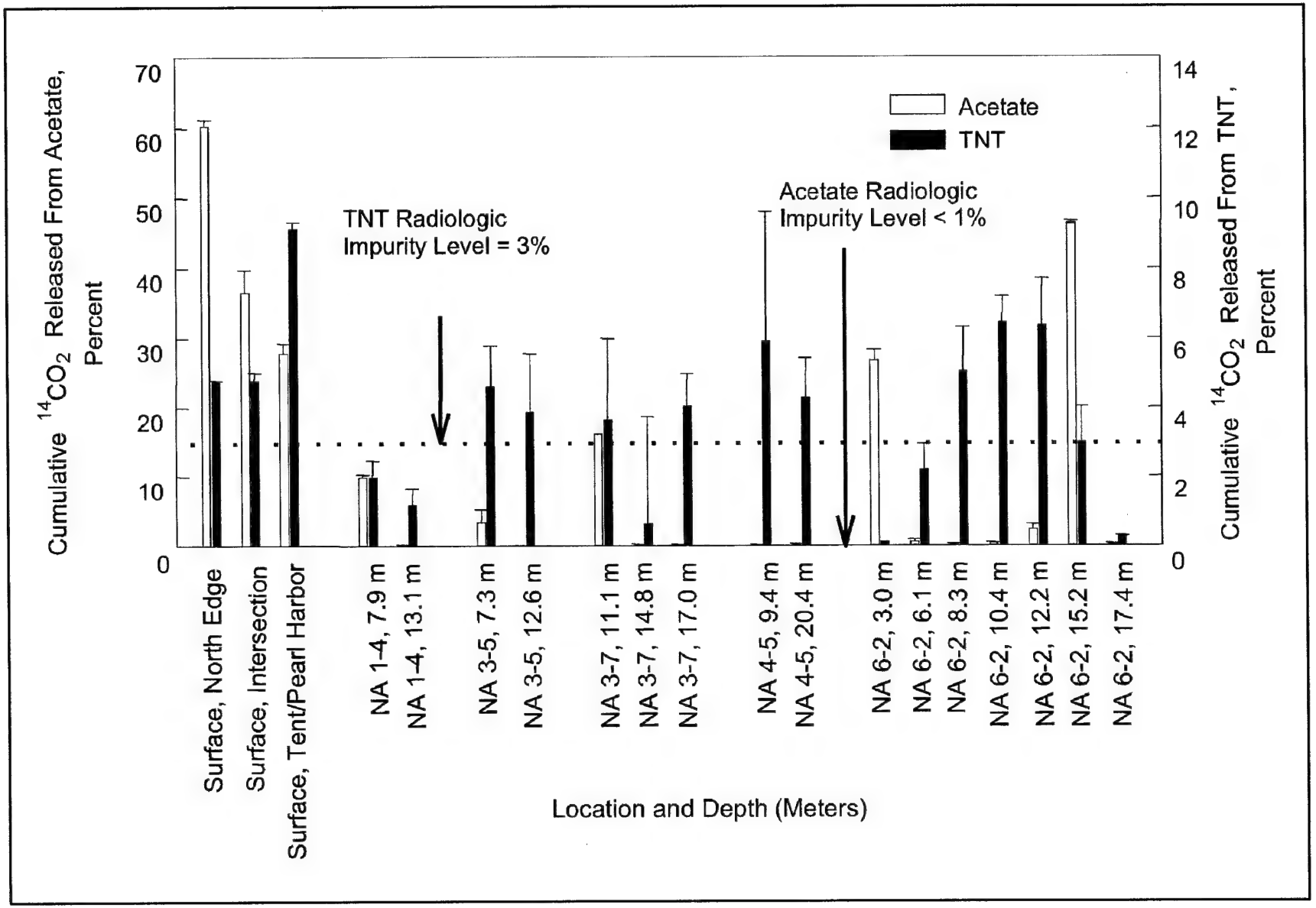


Figure 4. Comparison of total mineralization values for acetate and TNT obtained from LAAP surface and subsurface soils

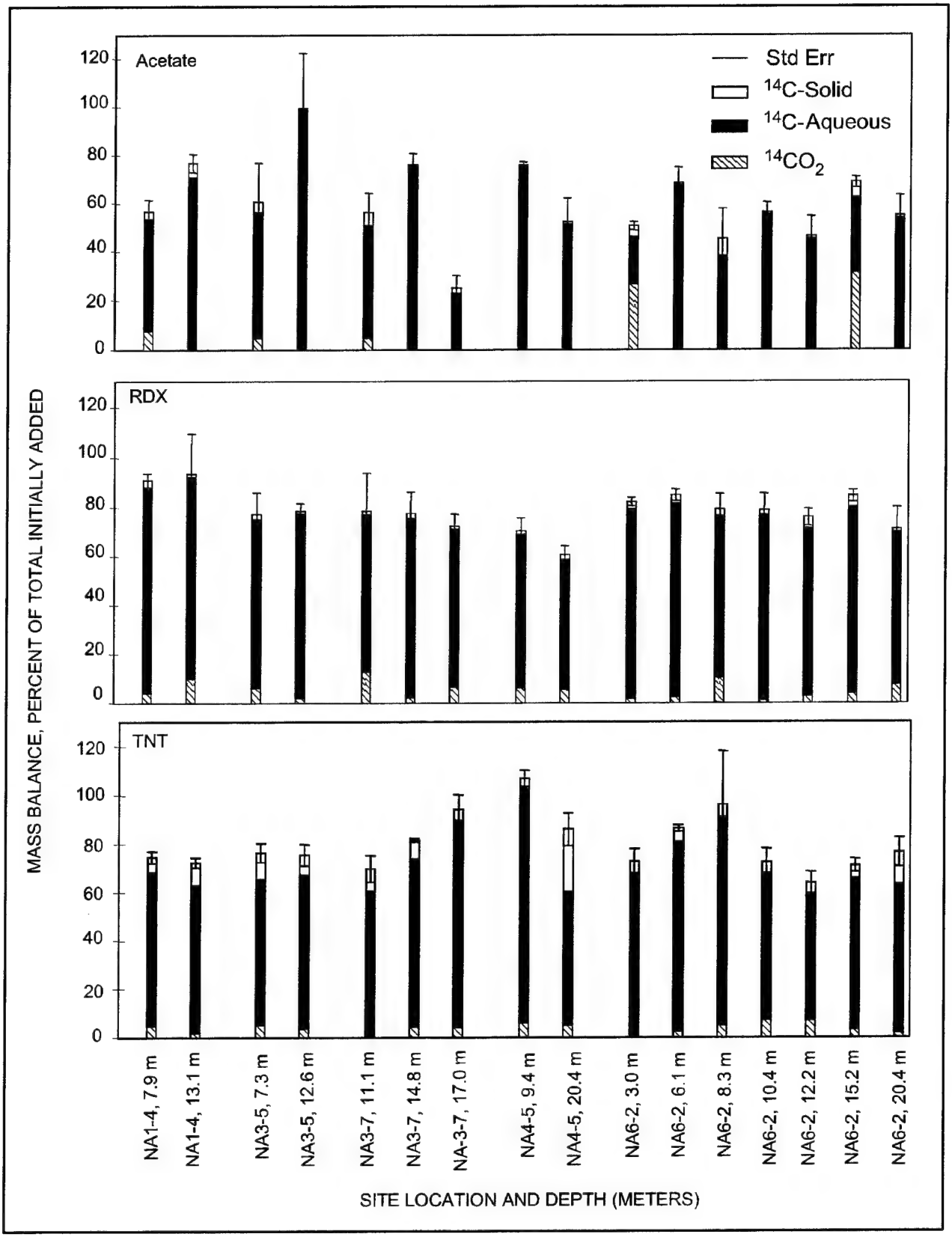


Figure 5. Mass balances for radiolabeled substrates used in LAAP microcosm studies

Genetic biomarkers

Isolation of DNA from soil cores. Soil samples from LAAP showed low biomass based on DNA yields. Total DNA yield ranged from below detection limit using SYBER green dye in agarose (<100-pg g^{-1} sediment) to 2-ng g^{-1} sediment (Table 5). The highest yields were associated with lines NA 1-4 and NA 2-1 at depths of 7-9 m.

Table 5
Yields of Total DNA and Estimated Biomass from LAAP Core Samples

Line	Depth, m	Avg. yield of DNA ng/g soil	Low Estimate of Number of Bacterial Cells per g of Soil[1]	High Estimate of Number of Bacterial Cells per g of Soil[2]
1-4	7.9	2.00	4.0E+5	1.3E+6
1-4	13.1	0.25	BD[3]	1.6E+5
2-1	7.9	1.36	2.7E+5	8.5E+5
2-1	10.4	BD[2]	BD	BD
2-1	14.3	0.30	6.0E+4	1.9E+5
3-5	7.3	0.45	9.0E+4	2.8E+5
3-5	12.5	0.82	1.6E+5	5.1E+5
3-7	10.7	BD	BD	BD
3-7	14.9	0.76	1.5E+5	4.8E+5
3-7	17.1	0.56	1.1E+5	3.5E+5
4-5	9.4	0.10	2.0E+4	6.2E+4
4-5	20.4	0.35	7.0E+4	2.2E+5
6-2	3.0	0.38	7.6E+4	2.4E+5
6-2	6.1	0.20	4.0E+4	1.3E+5
6-2	8.2	BD	BD	BD
6-2	10.4	0.10	2.0E+4	6.3E+4
6-2	12.2	0.10	2.0E+4	6.3E+4
6-2	15.2	0.20	4.0E+4	1.3E+5
6-5	17.4	0.12	2.4E+4	7.5E+4

[1] Based on 5 femtograms per cell.
[2] Based on 1.6 femtograms per cell.
[3] BD = Below detection limit.

Multiplex PCR analysis. Multiplex analyses were scored for the presence or absence of bands (Figure 2, Table 6). A band was registered as present if the band was observed at least twice in analysis of replicate subsamples. Based on previous examinations of aerobic, anaerobic, and microaerophilic TNT and dinitrotoluene-degrading microcosms, the biphenyl dioxygenase-related (245 bP) band is present only under anaerobic conditions (data not shown). The nitrotoluene/biphenyl

Table 6
Gene Biomarker Distribution in LAAP Soils

Target Gene	Core[1] Sample																
	1-4-7.9	1-4-13.1	2-1-3.4	2-1-7.8	2-1-14.3	3-5-7.3	3-5-12.5	3-7-10.7	3-7-14.9	3-7-17.1	4-5-9.4	4-5-20.4	6-2-3.0	6-2-6.1	6-2-8.2	6-2-10.4	6-2-15.2
Hydrogenase	0[2]	0	0	0	0	1[3]	1	0	0	0	0	0	0	1	0	0	1
NAD(P)H Flavin Nitroreductase	0	1	1	0	1	0	0	0	1	1	0	1	0	0	1	1	0
2-Nitrotoluene or Biphenyl dioxygenase	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
Biphenyl dioxygenase 285 bp	0	1	1	0	1	1	1	1	1	0	1	0	0	1	0	1	1
Biphenyl dioxygenase 275 bp	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0
"Anaerobic" Biphenyl dioxygenase 245 bp	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0
Biphenyl dioxygenase 220 bp	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0	1	1
Catechol-2,3-dioxygenase 195 bp	1	1	0	0	1	1	1	0	1	0	1	1	0	1	1	1	1
Catechol 2,3-oxygenase 150 bp	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1	0	1

(Continued)

[1] Sample depth in meters.
[2] No band detected.
[3] Band detected.
[4] ND = Not determined.

Table 6 (Concluded)

Target Gene	Core[1] Sample																
	1-4-7.9	1-4-13.1	2-1-3.4	2-1-7.8	2-1-14.3	3-5-7.3	3-5-12.5	3-7-10.7	3-7-14.9	3-7-17.1	4-5-9.4	4-5-20.4	6-2-3.0	6-2-6.1	6-2-8.2	6-2-10.4	6-2-15.2
Catechol-2,3-dioxygenase 135 bp	0	0	0	1	0	1	0	0	1	1	1	1	0	1	1	1	0
Catechol-2,3-dioxygenase 120 bp	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0
Catechol-2,3-dioxygenase 105 bp	1	0	0	0	1	1	0	1	0	0	0	1	1	0	0	0	0
Nitroreductase + Biphenyl dioxygenase 275 bp	0	0	0	0	ND[4]	0	0	0	0	0	0	1	0	0	1	0	0
Nitroreductase + Biphenyl dioxygenase 285 bp	0	1	1	0	ND	0	0	0	1	0	0	0	0	0	0	1	0
Biphenyl dioxygenase 245 bp + Biphenyl dehydrogenase 285 bp	0	0	0	0	ND	0	0	0	0	0	0	1	0	1	0	0	0
Hydrogenase + Biphenyl dioxygenase 285 bp	0	0	0	0	ND	1	1	0	0	0	0	0	0	1	0	0	1
C230 (150 or 195 bp) + (Nitroreductase or Biphenyl 245 bp) + (Biphenyl 275, 285, or 300 bp)	0	1	1	0	ND	0	0	0	1	1	0	1	0	1	1	1	0
C230 (120 or 135 bp) + (Nitroreductase or Biphenyl 245 bp) + (Biphenyl 275, 285, or 300 bp)	0	0	1	0	ND	0	0	0	1	1	0	1	0	1	1	1	0
Number of different C230 + (Nitroreductase or Biphenyl 245 bp) + (Biphenyl 245 bp) + (Biphenyl 285, 275, or 300 bp)	0	2	2	0	ND	0	0	0	2	2	0	3	0	2	4	2	0

dioxygenase (300 bp) and biphenyl dioxygenase (285 and 275 bp) genes were observed under either aerobic or microaerophilic (low-oxygen) conditions (data not shown). The nitroreductase primers amplify genes related to oxygen-sensitive nitroreductases. For purposes of relating genetic biomarkers to the conceptual pathway of Rieger and Knackmuss (1995; Figure 1), specific genes are considered indicators of either aerobic or anaerobic conditions. Nitroreductase and biphenyl dioxygenase-related (245 bp) bands were considered indicators of microaerophilic and anaerobic conditions, while nitrotoluene/biphenyl dioxygenase (300 bp) and biphenyl dioxygenase (285 and 275 bp) were considered indicative of aerobic conditions.

In general, multiplex PCR banding patterns were reproducible. Replicate extractions of core samples revealed some heterogeneity in distribution of microbes. Based on the data presented in Table 6, individual genes coding for the enzymes, in decreasing order of occurrence over the site (percent of total genes found), were as follows: catechol-2,3-dioxygenase-150 bp (16.5 percent) > catechol-2,3-dioxygenase-195 bp (13.9 percent) = biphenyl dioxygenase-285 bp (13.9 percent) > catechol-2,3-dioxygenase-135 bp (11.4 percent) > nitroreductase-300 bp (10.1 percent) > biphenyl dioxygenase (7.5 percent) = catechol oxygenase-105 bp (7.6 percent) > catechol-2,3-dioxygenase-120 bp (5.1 percent) > biphenyl dioxygenase-275 bp (3.8 percent) = biphenyl dioxygenase-245 bp (3.8 percent). The enzyme combinations most commonly found were C230-150 or 195 bp + nitroreductase. Thus, the site contained a high representation of genes needed to oxygenate aromatic compounds and reduce nitro groups, suggesting that the site has the genetic potential to transform explosives.

Genes detected from highly specific (stringent) primer sets (e.g., merRP, todC1, alkB, tmoA, dntAc, and ndoB) occurred sporadically within replicate subsamples. Thus, while genes associated with degradation of nitroaromatics in general were widely distributed over the site, more specialized genes coding for very specific portions of toluene metabolism were encountered much less frequently.

Membrane lipid biomarkers

Microbial biomass. Microbial abundance in subsurface soil at LAAP was one to two orders of magnitude less than results from nearer the surface (Table 7). Similar trends were observed with the DNA determinations of biomass (Table 5). The lipid biomarker analysis was able to describe a substantial biomass (i.e., above background) in most of the samples collected, allowing comparisons to be made with site geochemistry and contaminant distribution. Background PLFA levels (procedural artifacts) for the soils averaged 0.72 ± 0.68 pmole/g dry weight (1.8×10^4 cells/g dry weight) with a minimum value of 0.16 and a maximum of 1.6 ($n = 6$). After exclusion of all samples showing a PLFA concentration of less than 1.6 pmole/g, 90 percent of the sample pool remained for additional statistical evaluations. Estimates of microbial cell numbers were based on the assumption that 1 pmole of PLFA is equivalent to 2.5×10^4 cells of a typical subsurface bacterium (Balkwill et al. 1988).

Table 7
Compilation of Results for Lipid Biomarkers Extracted from Soil Samples at LAAP[1]

		Biomass		Community Composition (mole %)					Physiological Status (ratio)		Explosives Concentration ppb		Activity (% mineralization)		
Sample	Depth, m	pmole/g	cells/g[2]	Ubiquitous[3]	Gm + [4]	Gm -[5]	Actino, SRB, etc.[6]	Micro eukaryote[7]	Trans/cis[8]	Cyclopropyl/ monoenoic precursor[9]	RDX	TNT	Acetate	RDX	TNT
1-1	7.3	11.36	2.8E+5	78.6	0.00	14.36	0.00	6.88	0.34	0.00	0				
1-1	9.1	3.16	7.9E+4	59.77	0.00	20.64	0.00	19.59	0.00	0.00	0	0			
1-1	10.4	7.86	2.0E+5	49.91	1.06	33.06	0.00	15.97	0.36	0.00	206	81			
1-4	7.9	61.21	1.8E+6	21.19	25.11	27.42	22.72	22.72	0.00	4.07	84	40	9.8	ND[10]	ND
1-4	13.1	2.27	5.7E+4	57.14	0.00	9.16	0.00	33.71	0.00	0.00	0	0	0.1	6.0	ND
2-1	7.9	4.49	1.1E+5	42.05	0.00	57.95	0.00	0.00	0.00	0.00	3,080	1,840	2.5	0.5	ND
2-1	10.4	590	1.5E+5	78.76	0.58	17.82	0.00	2.83	0.00	0.20	3,910	1,140			
2-1	14.3	1.71	4.3E+4	80.13	3.94	13.88	0.00	2.06	0.00	0.00	1,690	1,660			
2-3	5.9	2.28	5.7E+4	73.80	3.74	20.08	0.00	2.37	0.00	0.00	0	0			
2-3	13.4	0.36	9.1E+3	87.50	0.00	12.50	0.00	0.00	0.00	0.00	0	0			
2-3	17.4	1.30	3.2E+4	88.65	0.00	11.35	0.00	0.00	0.00	0.00	308	219			
2-5	7.6	6.70	1.7E+5	73.00	4.93	19.97	0.00	2.10	0.33	0.00	0	0			
2-5	12.2	1.11	2.8E+4	92.47	0.00	7.53	0.00	0.00	0.00	0.00	0	0			
2-5	14.0	1.88	4.7E+4	86.64	0.00	13.36	0.00	0.00	0.00	0.00	0	0			
3-2	6.1	7.92	2.0E+5	75.88	0.00	22.38	0.00	1.74	0.00	0.00	0	0			

(Continued)

[1] Total viable biomass is estimated from PLFA concentration; community composition is derived from PLFA profiles, and physiological status is related to specific PLFA ratios. Explosive contaminant concentrations and microbial activities are provided for comparisons.
[2] Cell numbers are based on the assumption that 1 pmole is equivalent to 2×10^4 cells.
[3] Summation of normal saturated PLFA (e.g., 14:0, 15:0, 16:0, 17:0).
[4] Summation of terminally branched saturated PLFA (e.g., i15:0, a15:0, i16:0, i17:0, a17:0).
[5] Summation of monosaturated PLFA (e.g., 16:1w7cis, 18:1w7cis).
[6] Summation of branched saturated and monounsaturated PLFA (e.g., 10me16:0 and i17:1w7c).
[7] Summation of polyunsaturated PLFA (e.g., 18:2w6, 18:3w3, 20:4w6).
[8] Ratio of monounsaturated PLFA isomers (e.g., 16:1w7trans/16:1w7cis and 18:1w7trans/18:1w7cis).
[9] Ratio of cyclopropyl PLFA to their monounsaturated PLFA precursors (i.e., cyclopropyl 17:0/16:1w7cis and cyclopropyl 19:0/18:1w7cis).
[10] Not detected.

Table 7 (Concluded)

Sample	Depth, m	Biomass		Community Composition (mole %)					Physiological Status (ratio)		Explosives Concentration ppb		Activity (% mineralization)		
		pmole/g	cells/g[2]	Ubiquitous[3]	Gm + [4]	Gm -[5]	Actino, SRB, etc. [6]	Micro eukaryote[7]	Trans/cis[8]	Cyclopropyl/ monoenoic precursor [8]	RDX	TNT	Acetate	RDX	TNT
3-4	11.6	6.67	1.7E+5	67.76	0.00	32.24	0.00	0.00	0.00	0.00	0	0			
3-4	18.3	37.13	9.3E+5	89.91	0.00	10.09	0.00	0.00	0.00	0.00	0	0			
3-5	7.3	28.56	7.1E+5	43.19	0.00	27.78	0.00	29.02	0.00	0.00	0	0	3.3	0.5	1.6
3-5	12.8	7.14	1.8E+5	80.68	0.00	19.32	0.00	0.00	0.00	0.00	0	0	ND	ND	0.9
3-5	19.2	16.71	4.2E+5	81.11	0.00	18.89	0.00	0.00	0.00	0.00	0	0			
3-7	11.0	38.67	9.7E+5	63.87	5.39	26.67	0.74	3.32	0.50	1.56	0	0	16.0	42	ND
3-7	14.9	4.04	1.0E+5	82.10	2.55	13.87	0.00	1.48	0.36	0.00	0	0	ND	ND	0.6
3-7	17.1	66.26	1.7E+6	67.67	3.63	26.69	1.01	1.00	0.89	0.00	0	0	ND	3.4	1.3
4-5	9.4	3.22	8.1E+4	77.21	0.00	13.69	0.00	9.10	0.00	0.00	9,750	1,970	ND	ND	2.9
4-5	20.2	13.63	3.4E+5	31.20	14.35	30.87	7.44	16.31	0.00	1.90	1,990	1,380	ND	1.4	1.3
6-2	1.5	163.6	4.1E+6	23.73	17.94	34.56	16.63	7.14	0.09	1.07	0	0			
6-2	3.0	16.8	4.2E+5	57.62	8.19	25.56	2.31	6.32	0.36	2.26	0	0	26.8	ND	ND
6-2	4.6	17.4	4.3E+5	33.65	17.37	39.24	8.13	1.61	0.00	8.65	544	650			
6-2	6.1	0.6	1.4E+4	100.0	0.00	0.00	0.00	0.00	0.00	0.00	386	1,320	0.6	ND	ND
6-2	7.6	0.9	2.3E+4	49.65	2.26	36.09	0.00	12.00	0.02	8.17	789	3,050			
6-2	8.2	2.4	6.1E+4	75.85	0.00	24.15	0.00	0.00	0.00	0.00	4,360	5,120	0.1	5.7	2.0
6-2	9.1	1.8	4.4E+4	68.20	9.26	22.54	0.00	0.00	0.00	4.28	5,750	3,900			
6-2	10.4	0.6	1.6E+4	100.00	0.00	0.00	0.00	0.00	0.00	0.00	1,810	2,170	0.4	ND	3.4
6-2	12.2	11.9	3.0E+5	69.20	1.06	27.78	0.00	1.96	0.37	0.00	7,100	4,750	0.1	ND	3.4
6-2	13.1	1.3	3.2E+4	77.67	0.00	14.92	0.00	7.41	0.00	0.00	2,480	1,130			
6-2	13.7	2.9	7.2E+4	65.17	10.49	24.34	0.00	0.01	0.00	0.00	44	48			
6-2	15.2	3.4	8.6E+4	63.22	0.00	26.19	0.00	10.59	0.00	0.00	620	274	46.3	0.1	ND
6-2	17.4	9.8	2.5E+5	96.80	0.00	3.20	0.00	0.00	0.00	0.00	515	106	0.2	3.0	ND
6-2	18.3	5.0	1.2E+5	62.64	0.05	33.26	0.00	4.05	0.00	0.00	571	0			

Although microbial biomass decreased approximately one order of magnitude for every 3.0- to 6.1-m- (10- to 20-ft-) depth increment, no significant correlation between biomass and depth was observed. An inverse relationship between microbial biomass and the subsurface TNT concentrations was evident in one site transect, as shown in Figure 6 for Line 6-2, suggesting a negative impact of contamination on total microbial abundance. In general, the microbial population was metabolically active (viable) and possessed the genes needed for partial transformation of the nitroaromatic contamination.

Microbial community composition. Microbial communities were defined in terms of the relative percentages of individual PLFA present in each sample (i.e., a PLFA fingerprint). A hierarchical cluster analysis was used to evaluate similarities among the microbial community profiles. The test seeks relationships among cases (samples) given the types and magnitudes of the variables (PLFA) describing them. Results of the analysis are illustrated in Figure 7, wherein the occurrence of three distinct microbial communities has been identified. Taxonomic, functional, and contaminant characteristics of the three communities are provided in Table 8.

Two of the three communities identified in the cluster analysis contained samples with low-to-moderate levels of RDX and TNT contamination. Both communities showed a greater diversity of PLFA than that observed in the third community, which showed no TNT or RDX contamination in any of the samples. The presence of certain PLFA in nitroaromatic contaminated samples suggests that exposure to explosives has impacted the subsurface microbial ecology at LAAP. The induced microbial communities showed a greater relative abundance of gram-positive bacteria and other bacterial groups, such as the actinomycetes and the sulfate- and iron-reducing bacteria. These bacterial groups contain members able to play an integral role in the biodegradation of TNT.

The differences in microbial community composition can also be used to monitor progress in the natural selection process. Community 3, showing a substantial microeukaryotic abundance and simple bacterial profile, consists of samples exhibiting no detectable levels of nitroaromatic contamination. Most of the samples from this site were collected from area NA 3-7, which was outside of the zone of effect for the predicted subsurface contamination plume. The subsurface soils from this site reflect the naturally occurring microflora associated with the local biogeochemical environment. By contrast, the microbial composition of samples comprising Community 2 reveals impacts of the subsurface contamination. These two distinct communities can be represented graphically using a principal-components analysis (Figure 8). The analysis reduces between-sample variance by placing as much of the variance as possible into single factors. A plot can then be generated that places the two distinct microbial communities in a definable two-dimensional space. PLFA results from the collection of samples for any future analysis—1 to 5 years into the future—can then be added to the plot. Progress by in situ contaminant-associated microflora toward development of a community profile characteristic of the naturally occurring (noncontaminant associated) microflora can then be measured.

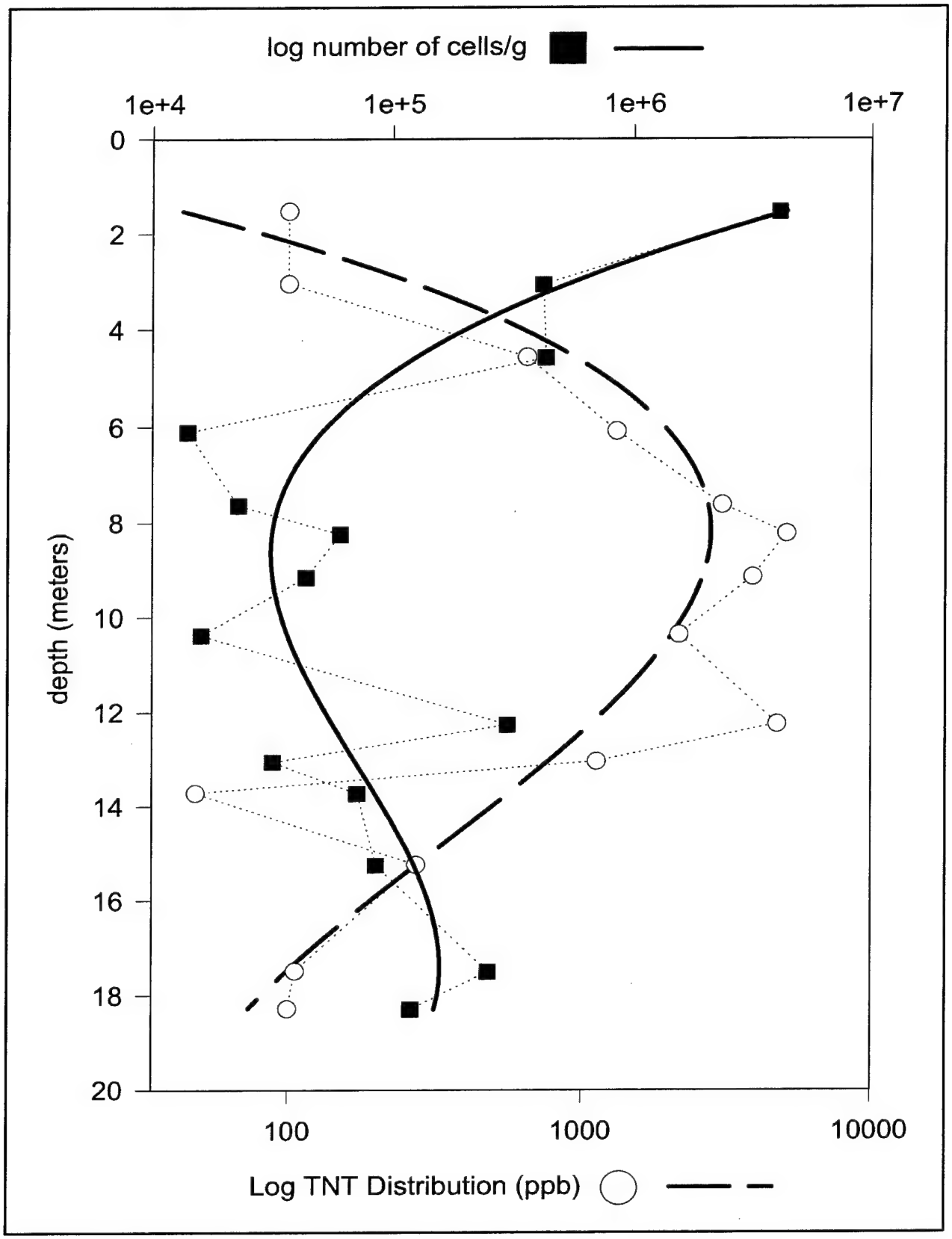


Figure 6. Comparison of TNT distribution and viable biomass (PLFAME) in Soil Core 6-2

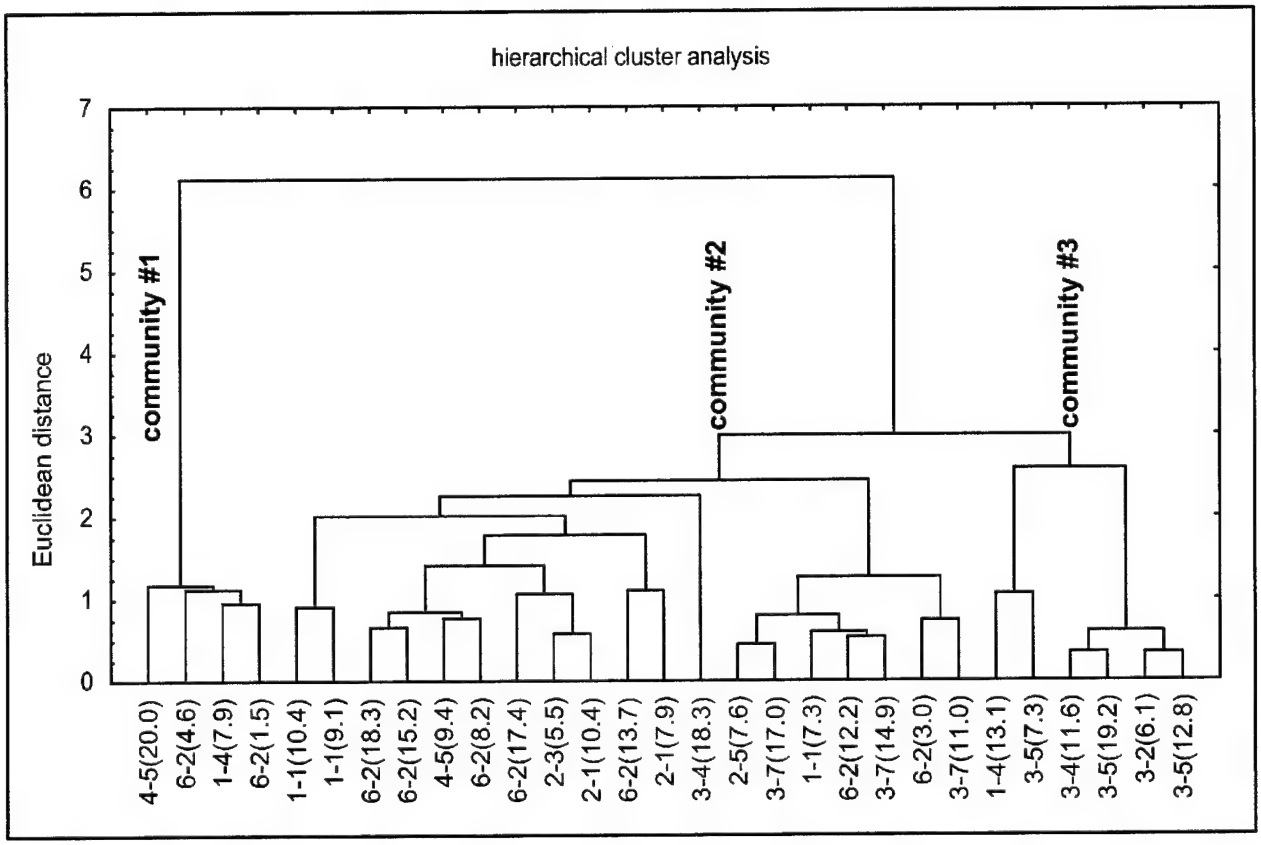


Figure 7. Dendogram depicting relationship between cluster groups defined by membrane lipid biomarkers

Interrelationships between biomarkers and contaminant concentration, extent of contaminant mineralization and biogeochemistry

The subsurface microbiota at LAAP were assayed for the following characteristics: (a) ability to mineralize acetate, RDX, and TNT; (b) absence or presence of functional genes related to known or assumed explosives degradation pathways; (c) presence or magnitude of a viable microbial biomass; and (d) composition of the viable microbial community. Technologies applied in determining these characteristics were radiorespirometry microcosms, multiplex PCR of recovered nucleic acids, and membrane lipid biomarker analysis. The results of each assay were related to subsurface soil concentrations of RDX and TNT and to mineralization rates for each explosives contaminant. To identify those factors from each assay that were correlated to natural attenuation (i.e., contaminant mineralization and concentration), a matrix of all results was generated and evaluated using the Spearman Rank Order Correlation. Significant results of this analysis are presented in Table 9.

Table 8
Functional Characteristics and Contaminant Concentrations Associated with Three Microbial Communities Defined by Hierarchical Cluster Analysis

	Community No. 1		Community No. 2		Community No. 3	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Microbial Biomass (cells/g)						
Cells/g	1.6E + 06	1.7E + 06	3.2E +05	4.2E + 05	2.9E + 05	2.4E + 05
Microbial Community Composition (mole%)						
Ubiquitous	27.44	5.93	69.86	13.19	67.63	15.02
Gram-positive	18.69	4.56	2.19	3.12	0.00	0.00
Gram-negative	33.02	5.07	23.12	11.43	21.63	7.99
Other bacterial	13.73	7.31	0.21	0.58	0.00	0.00
Microeukaryotic	7.15	6.52	4.61	5.63	10.75	16.01
Microbial Physiological Stress (ratios, as given in footnotes)						
Long-term stress[1]	0.02	0.05	0.18	0.25	0.00	0.00
Short-term stress[2]	3.92	3.40	0.21	0.61	0.00	0.00
Contaminant Concentrations (ppm)						
RDX	655	922	1,587	2,813	0	0
TNT	518	647	807	1,582	0	0

[1] Trans/cis isomers.
[2] Cyclopropyl/monoenoic precursors.

Rates of RDX and TNT mineralization were very low in LAAP soils (range of below detection to 0.214 percent per day for RDX; below detection to 0.219 percent per day for TNT), and only a few correlations with the biomarker parameters were identified. RDX mineralization correlated positively with a single PCR product, catechol-2,3-dioxygenase-150 bp. TNT mineralization rates correlated positively with another catechol oxygenase gene.

Similar results were observed between biomarkers and contaminant concentrations. The two catechol oxygenase gene probes, showing significant correlations with TNT and RDX mineralization rates, also correlated positively with HMX, RDX, and TNT concentrations. These probes indicate the potential for the aerobic degradation of TNT. Positive identification of the catechol probes is strong evidence that microbial populations contain at least some, if not all, of the necessary genes for explosives mineralization.

The lack of substantial levels of organic carbon at LAAP could be another factor limiting microbial abundance and degradative capability (i.e., cometabolism). The subsurface soil at LAAP consists of 84-percent sand with a relatively low sorption capacity for nitroaromatic compounds (0.04 to 0.33 L/kg). In the absence of sorption, more of the compound will remain in solution, potentially exerting

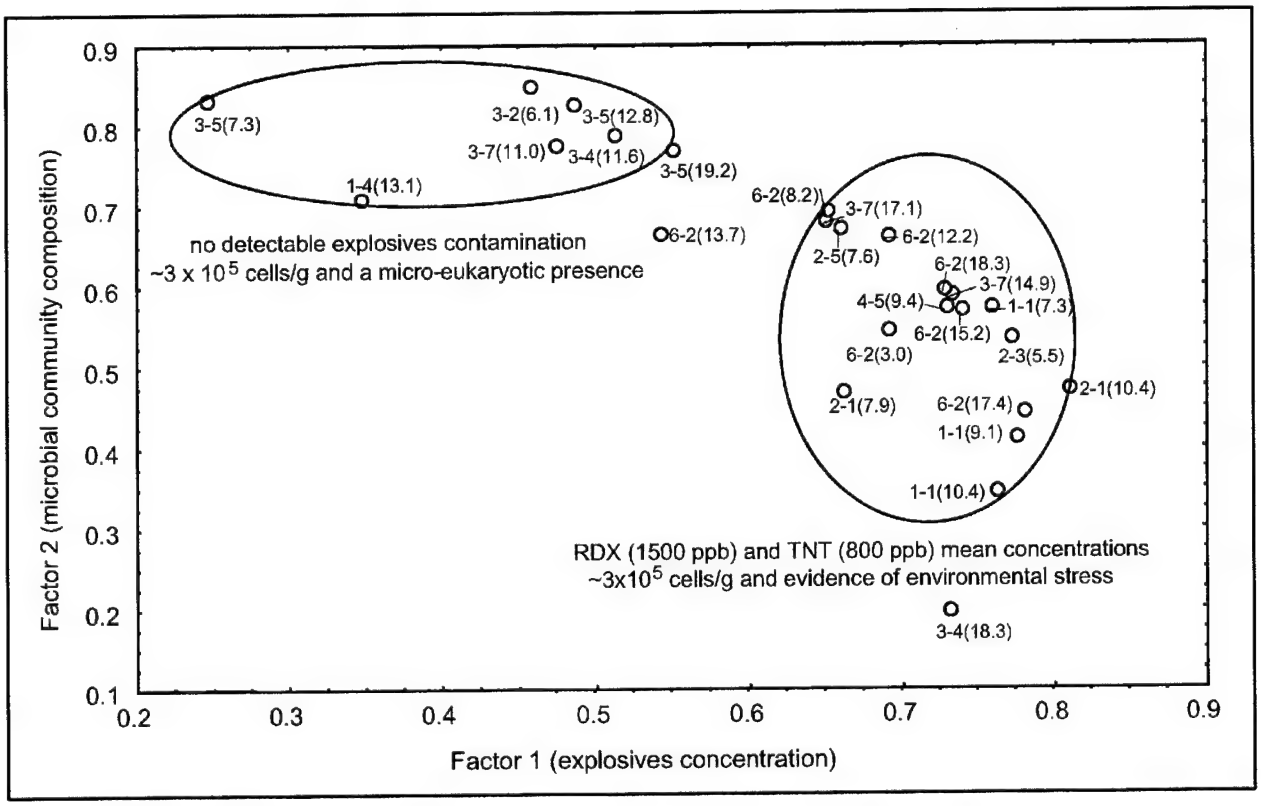


Figure 8. Principal-components analysis describing relationships between membrane lipid biomarker components and explosives concentration for LAAP natural attenuation study

toxicity towards the native microflora. The negative correlation between microbial biomass and soil TNT concentrations suggests such a toxicity effect.

Results of the LAAP study indicated a potential for microbial degradation of nitroaromatic contaminants in the subsurface at the LAAP site. The degradative potential measured ex situ (i.e., via radiorespirometry) was verified in situ by the application of the two biomarker tools. A genetic capacity for aerobic biodegradation of TNT, although limited, was identified in a subset of the indigenous microbial population. The presence of nitroaromatic contamination induced a significant change in the composition of the subsurface microflora, thus providing a benchmark for tracking the progress of natural attenuation at LAAP.

Results for Joliet Army Ammunition Plant (JAAP)

Radiorespirometry

Mineralization of acetate and explosives. Acetate mineralization in JAAP soils was moderate, generally 20-40 percent, indicating the presence of active microbial populations in all samples (Table 10, Figure 9).

Table 9
Spearman Rank Order Correlations for Mineralization Rates, Contaminant Concentrations, Geochemistry, and Microbial Biomarkers Found at LAAP

Mineralization Rates			Contaminant Concentrations					Geochemistry		Genetic Biomarkers				Membrane Lipid Biomarkers					
ACErate	RDXrate	TNTrate	HMX	RDX	TNB	TNT	2ADNT	%SILT	TOC	Hyd	Nred	C23O	C23O 125 bp	Biomass	Gram (+)	Gram (-)	Actino.	ST-stress	ST-stress
ACErate	0.09	-0.53[1]	-0.43	-0.27	-0.31	-0.27	-0.32	0.17	-0.16	0.02	-0.54	-0.72	-0.17	0.35	0.41	0.26	0.56	0.66	0.56
	RDXrate	-0.06	-0.13	-0.11	-0.18	-0.03	-0.07	0.04	0.32	-0.17	0.38	-0.11	0.40	0.08	-0.03	0.27	-0.01	0.01	-0.01
		TNTrate	0.75	0.63	0.62	0.69	0.49	-0.60	0.23	-0.13	0.31	0.65	0.33	-0.12	-0.23	0.18	-0.30	-0.35	-0.30
			HMX	0.90	0.93	0.92	0.75	-0.29	0.24	-0.31	0.26	0.47	0.59	-0.48	-0.25	-0.11	-0.26	-0.30	-0.26
				RDX	0.96	0.91	0.70	-0.26	0.39	-0.20	0.20	0.41	0.50	-0.41	-0.29	0.00	-0.29	-0.34	-0.29
					TNB	0.94	0.77	-0.23	0.31	-0.13	0.20	0.44	0.43	-0.53	-0.33	-0.18	-0.32	-0.37	-0.32
						TNT	0.69	-0.30	0.30	-0.17	0.20	0.53	0.50	-0.52	-0.29	0.00	-0.26	-0.31	-0.26
							2ADNT	0.13	0.53	-0.11	0.25	0.38	0.36	-0.39	-0.22	-0.19	-0.32	-0.32	-0.32
								%SILT	-0.11	0.23	-0.07	-0.16	0.14	-0.20	-0.10	-0.24	0.06	0.09	0.06
									TOC	-0.57	0.70	0.26	0.08	0.24	0.33	0.23	0.00	-0.05	0.00
										Hyd	-0.52	-0.03	-0.31	-0.19	0.54	-0.13	-0.33	-0.33	-0.33
											Nred	0.18	0.31	-0.10	0.19	-0.07	-0.20	-0.27	-0.20
												C23O	-0.03	-0.15	-0.18	0.20	-0.34	-0.41	-0.34
													C23O 125 bp	-0.26	-0.28	0.09	-0.27	-0.27	-0.27
														Biomass	0.70	0.67	0.56	0.56	0.56
															Gram (+)	0.45	0.80	0.77	0.80
																Gram (-)	0.40	0.35	0.40
																	Actino.	0.97	1.00
																		ST-stress	0.97
																			ST-stress

[1] Shaded cells highlight coefficients of 0.5 or greater, which are significant at a 95-percent confidence level.

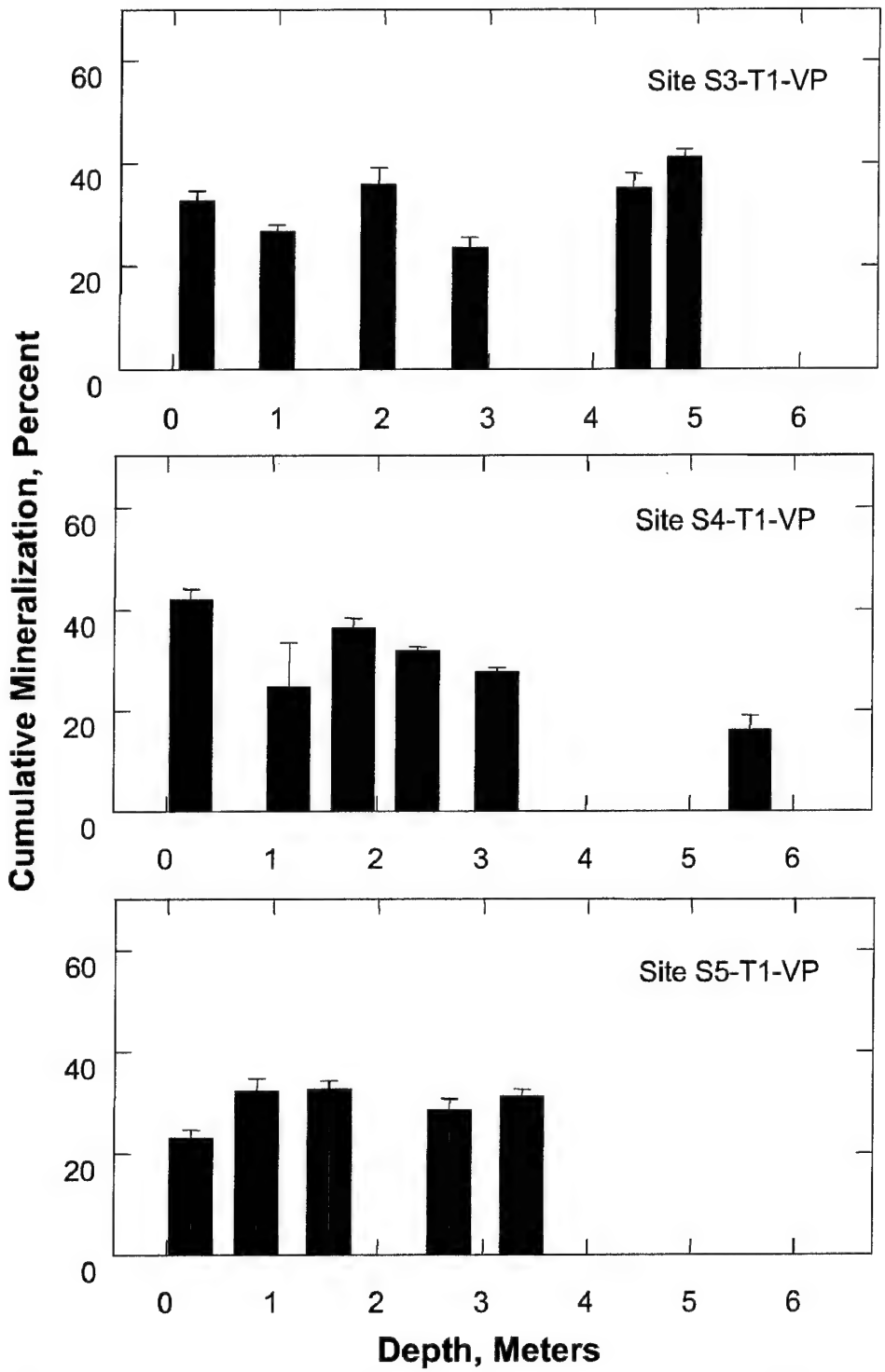
Table 10
Mineralization Rates for Joliet Army Ammunition Plant Soil Samples Taken with Depth at Each of the Three Profile Locations[1]

JAAP Sample Profile	Depth, m	Acetate Rate %/day ± S.E.[2]	RDX Rate %/day ± S.E.[3]	TNT Rate %/day ± S.E.[3]
Site S3-T1-VP	0.23	6.52 ± 0.39	0.238 ± 0.018	0.128 ± 0.026
	0.99	5.34 ± 0.26	0.043 ± 0.007	0.038 ± 0.007
	1.95	7.16 ± 0.66	0.130 ± 0.013	ND[4]
	2.80	4.70 ± 0.41	0.073 ± 0.008	ND
	4.34	7.04 ± 0.55	0.111 ± 0.005	ND
	4.91	8.22 ± 0.30	0.070 ± 0.026	ND
Site S4-T1-VP	0.23	8.42 ± 0.42	ND	0.389 ± 0.010
	1.14	4.96 ± 1.75	0.126 ± 0.074	0.264 ± 0.026
	1.81	7.30 ± 0.37	0.163 ± 0.018	0.009 ± 0.021
	2.39	6.40 ± 0.13	0.110 ± 0.023	ND
	3.14	5.54 ± 0.17	0.039 ± 0.006	ND
	5.56	3.24 ± 0.55	0.054 ± 0.009	ND
Site S3-T2-VP	0.23	4.62 ± 0.33	0.184 ± 0.027	0.030 ± 0.010
	0.87	6.46 ± 0.49	0.178 ± 0.005	0
	1.54	6.54 ± 0.31	0.156 ± 0.020	0.021 ± 0.021
	2.66	5.72 ± 0.43	0.090 ± 0.008	ND
	3.37	6.24 ± 0.39	0.305 ± 0.012	ND

[1] Values given are the means of three replicates ± standard error of the mean. All tests were run at 23.3 ± 3.2 °C.
[2] Acetate rates determined over 5 days.
[3] RDX and TNT rates determined over 28 days.
[4] ND indicates that the total level of mineralization did not exceed 3 percent over the 5- (acetate) or 28-day (RDX and TNT) incubation period.

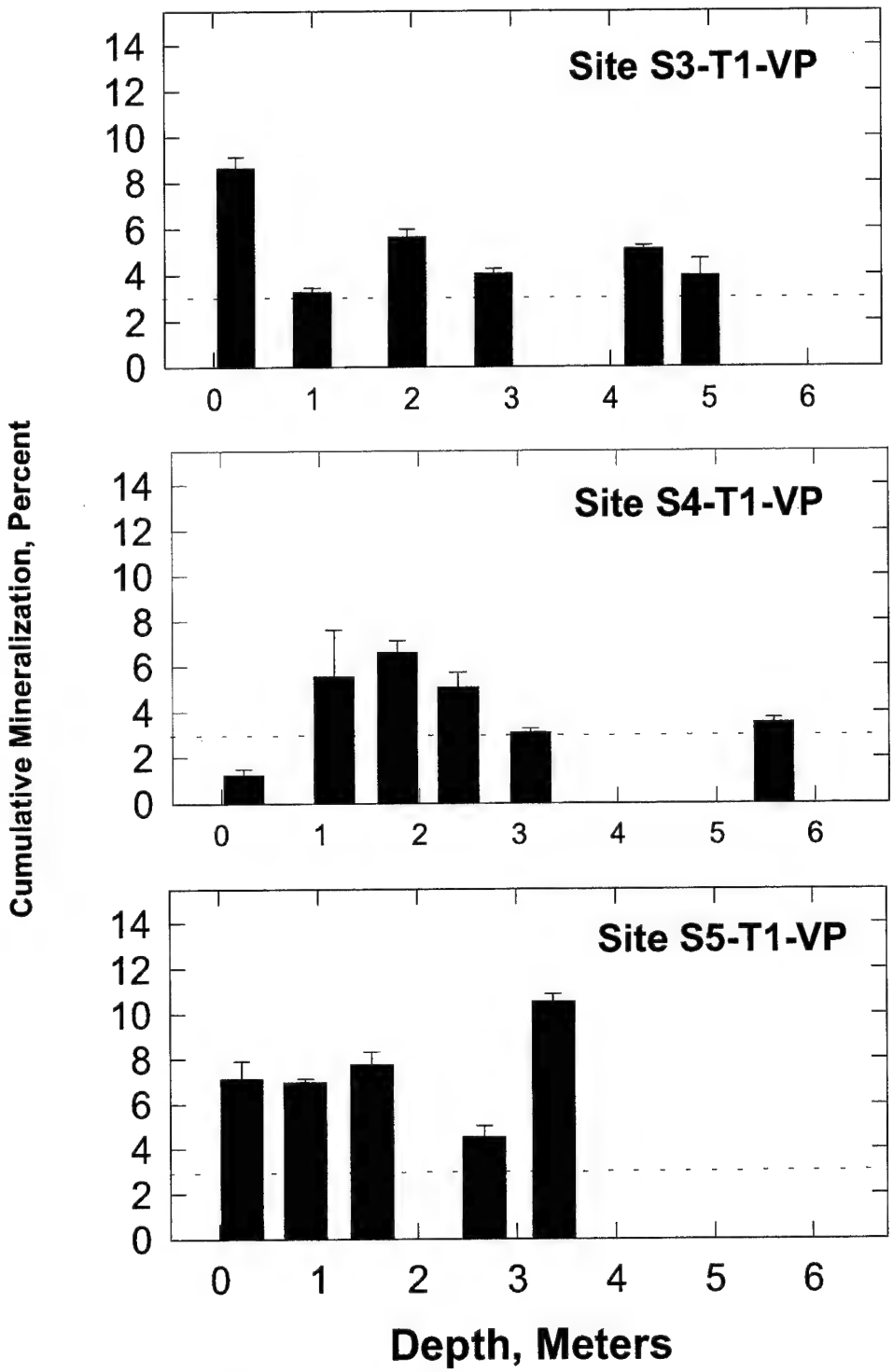
Resident microbial communities exhibited the potential to mineralize RDX in the field showing low-to-moderate levels of mineralization to occur over the 28-day incubation period (Figure 10 and Table 10). The standard error values for total RDX mineralization in the subsurface were generally small (at or less than 10 percent of the mean).

Mineralization of TNT by resident microbial communities was limited, ranging from <1 percent to a maximum of 14 percent in a near-surface soil from Site S4-T1 (corrected for level of impurities) (Table 10, Figure 11). Sites S3-T1 and S3-T2 also showed the greatest mineralization extent near the surface. Most TNT



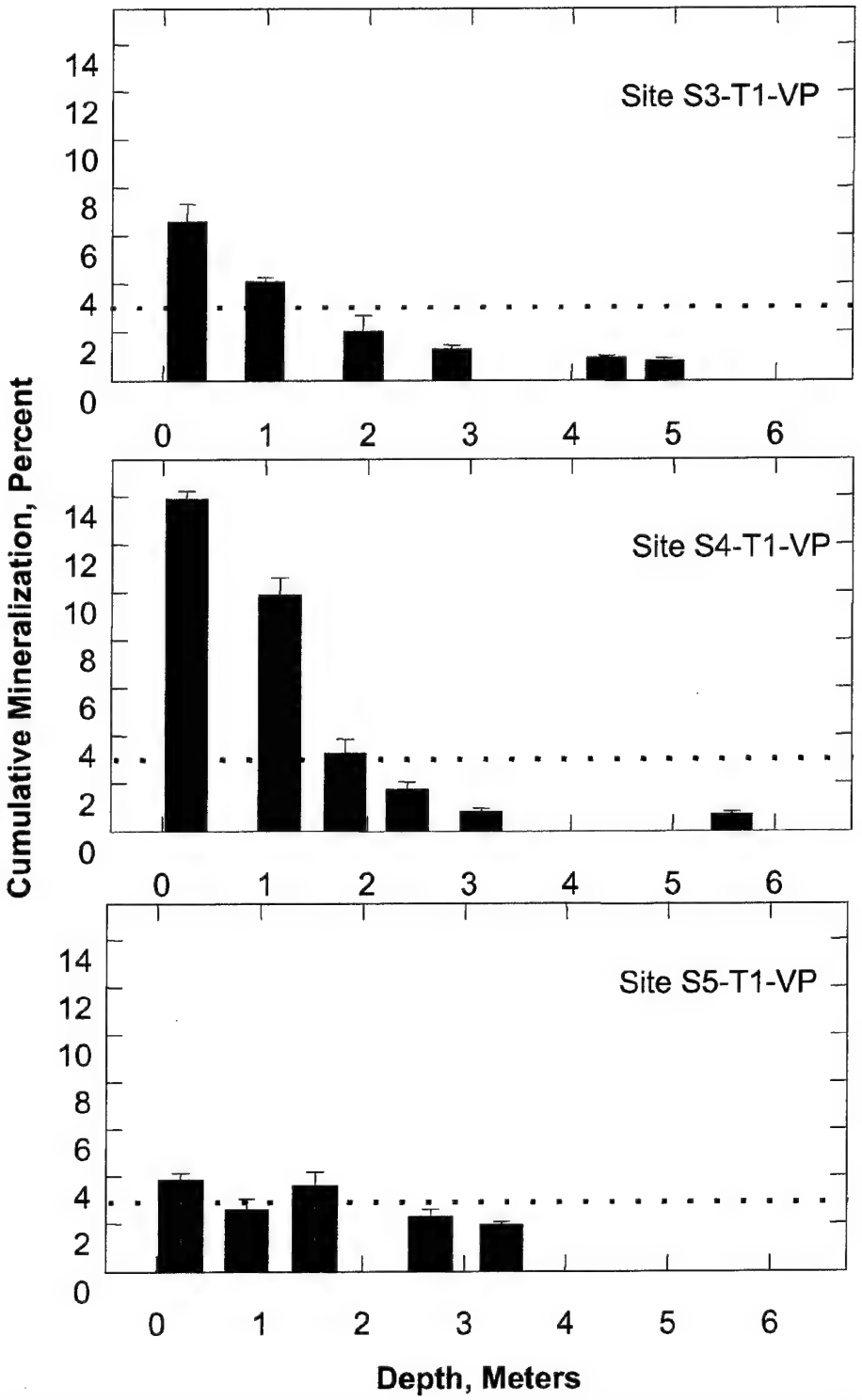
*Values given are the means of three replicates plus or minus the standard error of the mean for 5 days of incubation.

Figure 9. Acetate mineralization in Joliet vertical profile samples (Radiochemical purity was >99 percent)



Values given are the means of three replicates ± standard error for 28 days of incubation.
Dashed lines in each panel represent the level of impurities present in the radiolabeled RDX.

Figure 10. RDX mineralization in Joliet vertical profile samples



Values given are the means of three replicates ± standard error for 28 days of incubation.
Dashed lines in each panel represent the impurity level present in the radiolabeled TNT.

Figure 11. TNT mineralization in Joliet vertical profile samples

mineralization rates for the three profile soils were slightly higher than rates from LAAP soils, but typically less than 0.100 percent day^{-1} (Table 10). Values at or in excess of 0.200 percent day^{-1} tended to occur at the surface or within 0.61 m of the surface (Figure 11, Table 10). The distributions of TNT mineralization at all three core locations showed a pattern of decreasing activity with depth, which corresponds to the contaminant distribution (Figure 11).

Mass balances for acetate and explosives mineralization. Total recoveries of ^{14}C originally added as acetate to subsurface soil averaged 82 percent (Figure 12, upper panel). Total $^{14}CO_2$ was generally between 25 and 40 percent of the total amount added (Figure 12, upper panel). Most (30-65 percent) of the radioactivity was found in the solid phase with little or no accumulation in the aqueous phase. Standard errors for the total mass balances were generally less than 10 percent of the mean.

Total ^{14}C recoveries from most of the soil segments treated with radiolabeled RDX (Figure 12, middle panel) were at or above 80 percent, with an average total recovery of 89 percent. Most of the radioactivity was split equally between the aqueous and solid phases; only low-to-moderate amounts (0 to 7.5 percent, corrected for impurities) were recovered as $^{14}CO_2$.

Mass balances for radiolabeled TNT ranged from 79-119 percent (average of 91 percent) (Figure 12, bottom panel). Most of the activity was found in the aqueous phase of the respirometers. Very little (<1 percent) radioactivity was recovered as $^{14}CO_2$.

Genetic biomarkers

Multiplex PCR analysis. RDX and TNT contamination was sometimes found in the uppermost layers near the soil surface, but was not found in soil profile samples taken well below the surface. Nonetheless, genes related to biphenyl extradiol dioxygenases (bph) and NAD(P)H nitroreductases (nreduct), *Desulfovibrio* sp. dissimilatory sulfite reductases (dsrB), and catechol-2,3-dioxygenases (xylE-type c230) were found in each vertical soil profile. Figure 13 depicts the genes present in a typical sample from one section of a soil profile. The presence of genes related to the Tn501mercury resistance marker gene (merRP), iron-sulfur alpha subunit of toluene dioxygenase (todC1), toluene-4-monooxygenase (tmoA), alkane hydroxylase (alkB), ammonia monooxygenase (amoA), or sigma 54-dependent activators was not detected.

The relative abundance of target genes for the three profile sites at JAAP is shown in Table 11. The genes most frequently encountered, in decreasing order of occurrence (percent of total genes found), were catechol-2,3-dioxygenase (26.5 percent) > nitroreductase (20.6 percent) > biphenyl dioxygenase (17.6 percent) > anaerobic biphenyl dioxygenase-250 bp (11.8 percent). The most frequently encountered combinations of genes were nitroreductase or sulfite reductase with either biphenyl dioxygenase-275 bp or C230 and nitroreductase-380 bp with biphenyl dioxygenase-275 bp. Similar to LAAP, JAAP soils had abundant and widely distributed levels of genes anticipated to participate in the

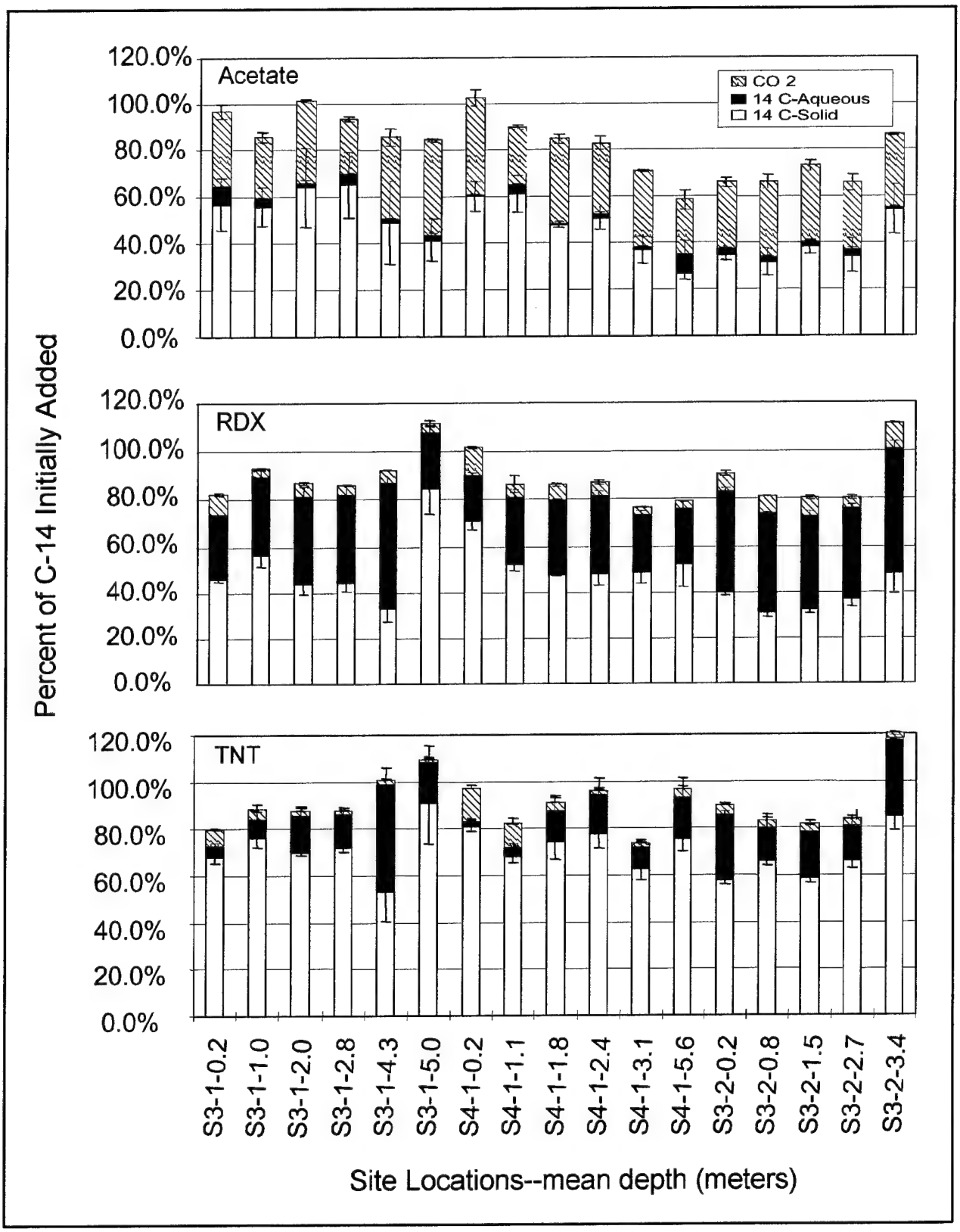


Figure 12. Mass balances for acetate, RDX, and TNT from vertical profile Sites S3, S4, and S5 at JAAP

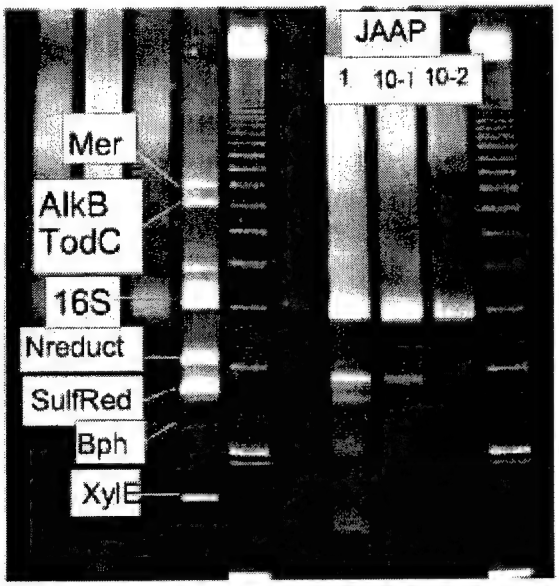


Figure 13. Detection of degradative genes in JAAP explosives-contaminated surface soils (Dilutions of DNA were examined for presence of degradative genes using multiplex PCR)

degradation of nitroaromatic explosives (Figure 1). In addition, the finding at JAAP of both aerobic and anaerobic biphenyl dioxygenase genes and the presence of sulfite reductase indicates that both anaerobic and aerobic conditions may be important for TNT degradation in JAAP soils.

Membrane lipid biomarkers

Microbial biomass. At the JAAP site, the contamination was concentrated near the surface (0-0.61 m) (Chapter 2, Table 19, VP samples). Of the 17 samples collected for microbial activity, only 2 showed detectable levels of TNT, and none showed the presence of RDX. JAAP subsurface microbial biomass averaged 10^6 cells g^{-1} dry weight of soil, which was two orders of magnitude less than surface measurements. Biomass decreased linearly with increasing depth at Sites 3-1 and 4-1, but remained relatively constant throughout the depth profile at Site 3-2 (Figure 14).

Microbial community composition. Two distinct microbial communities were identified at JAAP using lipid biomarkers. One community occurred in samples collected from depths of 1.83 m or less, and the other was found in samples obtained from depths greater than 1.83 m. The exceptions to this pattern were two shallow cores collected from Site 3-1. This result is comparable with other evaluations of subsurface microbial ecology, where populations decline with depth (White and Ringelberg 1997).

The three sites at JAAP exhibited differences in surface contamination. Site 3 was located next to a ridge and furrow area having high levels (1,000-4,000 $mg \cdot kg^{-1}$) of TNT contamination. Microbial community patterns also differed among the three depth profiles (Figure 15). Surface cores taken at Sites 3-1 and 4-1 showed a predominance of gram-negative lipid biomarkers and substantial percentages of bacterial lipids similar to sulfate-reducing bacteria and microeukaryotes (i.e., fungal). The surface core from Site 3-2 showed a predominance of actinomycete lipid biomarkers, very little evidence for sulfate-reducing bacteria, and no microeukaryotic presence. The differences in surface microbial community composition is probably a function of the local contaminant and geochemical characteristics of the soil.

Table 11
Genetic Biomarker Distribution in JAAP Soils

Target Gene		Sample																
		S3T1 - VP1	S3T1-VP2	S3T1-VP3	S3T1-VP4	S3T1-VP5	S3T1-VP6	S4T1-VP2	S4T1-VP3	S4T1-VP4	S4T1-VP5	S4T1-VP6	S4T1-VP7	S3T2-VP2	S3T2-VP3	S3T2-VP4	S3T2-VP5	S3T2-VP6
NAD(P)H Flavin nitroreductase	380 bp	0[1]	1[2]	0	0	0	0	1	1	1	0	1	0	0	1	1	0	0
Dissim. sulfite reductase	320 bp	0	1	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0
Biphenyl dioxygenase	275 bp	1	1	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0
"Anaerobic" biphenyl dioxygenase	250 bp	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
Unknown band	225 bp	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Catechol -2,3-dioxygenase	185 bp	1	0	0	0	1	0	1	1	1	1	1	0	0	0	0	0	0
Atrazine reductase	150 bp	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Catechol -2,3-dioxygenase	125 bp	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Presence of nitroreductase or sulfite reductase with either bph dioxygenase or c23o	nred/dsrb +bph/c23o	0	1	0	0	1	0	1	1	1	0	1	0	0	0	0	0	0
Total number of different genes or biomarkers	total	5	3	0	0	2	0	7	4	5	1	4	0	1	1	1	0	0
Presence of nitroreductase with bph dioxygenase	nred/ +bph	0	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0

[1] 0 = Target gene not found.
[2] 1 = Target gene found.

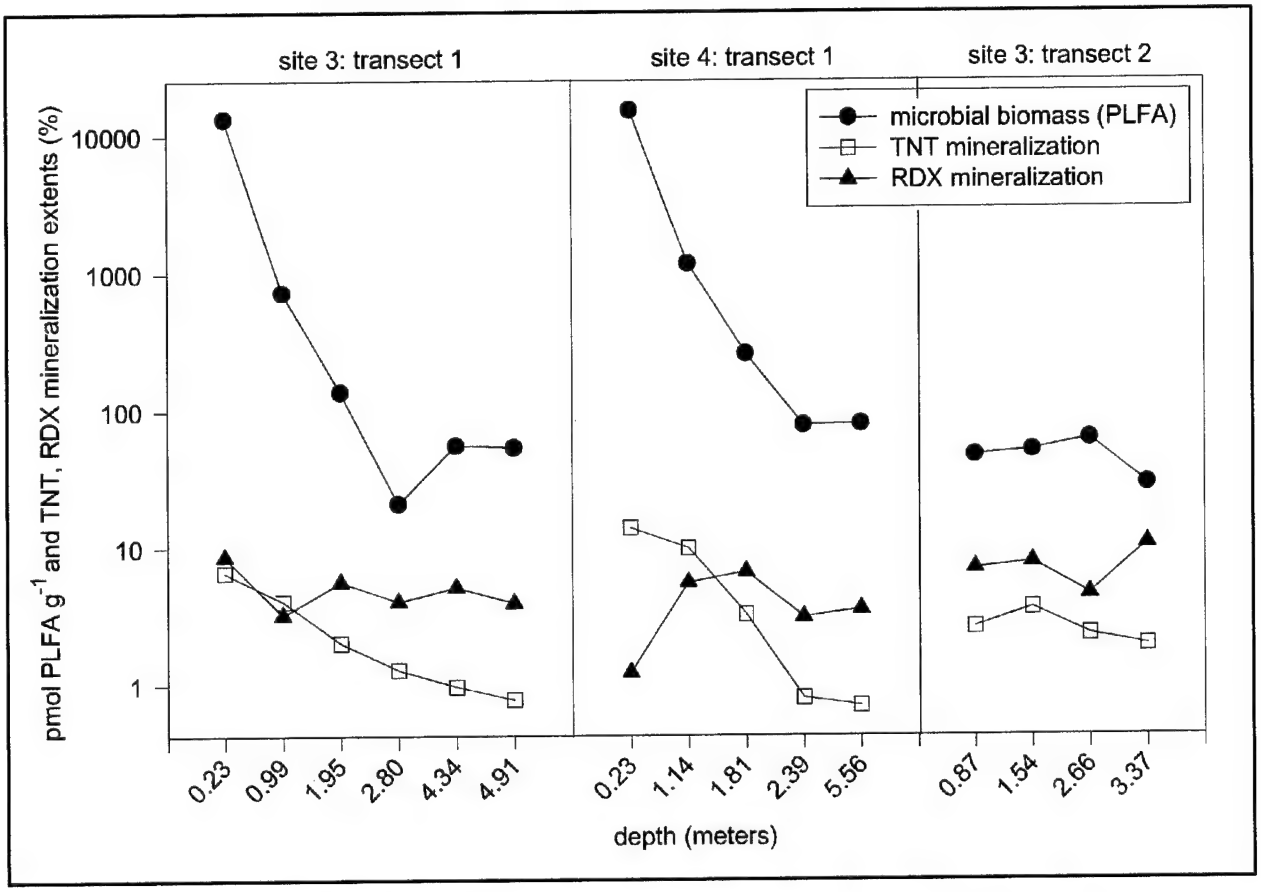


Figure 14. Microbial biomass estimates and mineralization extents for TNT and RDX in JAAP soils

Interrelationships between biomarkers and contaminant concentration, extent of contaminant mineralization and biogeochemistry

Several biomarker parameters were significantly correlated to ex situ rates for TNT mineralization, while rates of RDX mineralization were not significantly correlated with any of the measured biomarkers. TNT mineralization rates correlated with both nucleic acid and membrane lipid biomarkers. TNT concentrations in the surface and subsurface soils also correlated positively with the presence of two reductive transformation products of TNT, 2ADNT and 4ADNT.

Analysis of the biomarker results from JAAP revealed that factors different from those derived from LAAP were related to TNT mineralization. The results for both LAAP and JAAP demonstrated that each assay supplied a distinct perspective on the subsurface biota and its interactions with the contaminants. To identify those factors that were relevant to contaminant mineralization (i.e., natural attenuation), a matrix was developed and compared using a Spearman Rank Order Correlation (Table 12). TNT mineralization rates correlated positively with three of the four nucleic acid probes, the membrane lipid biomarker measure of microbial biomass, and estimates of gram-negative and sulfate- and

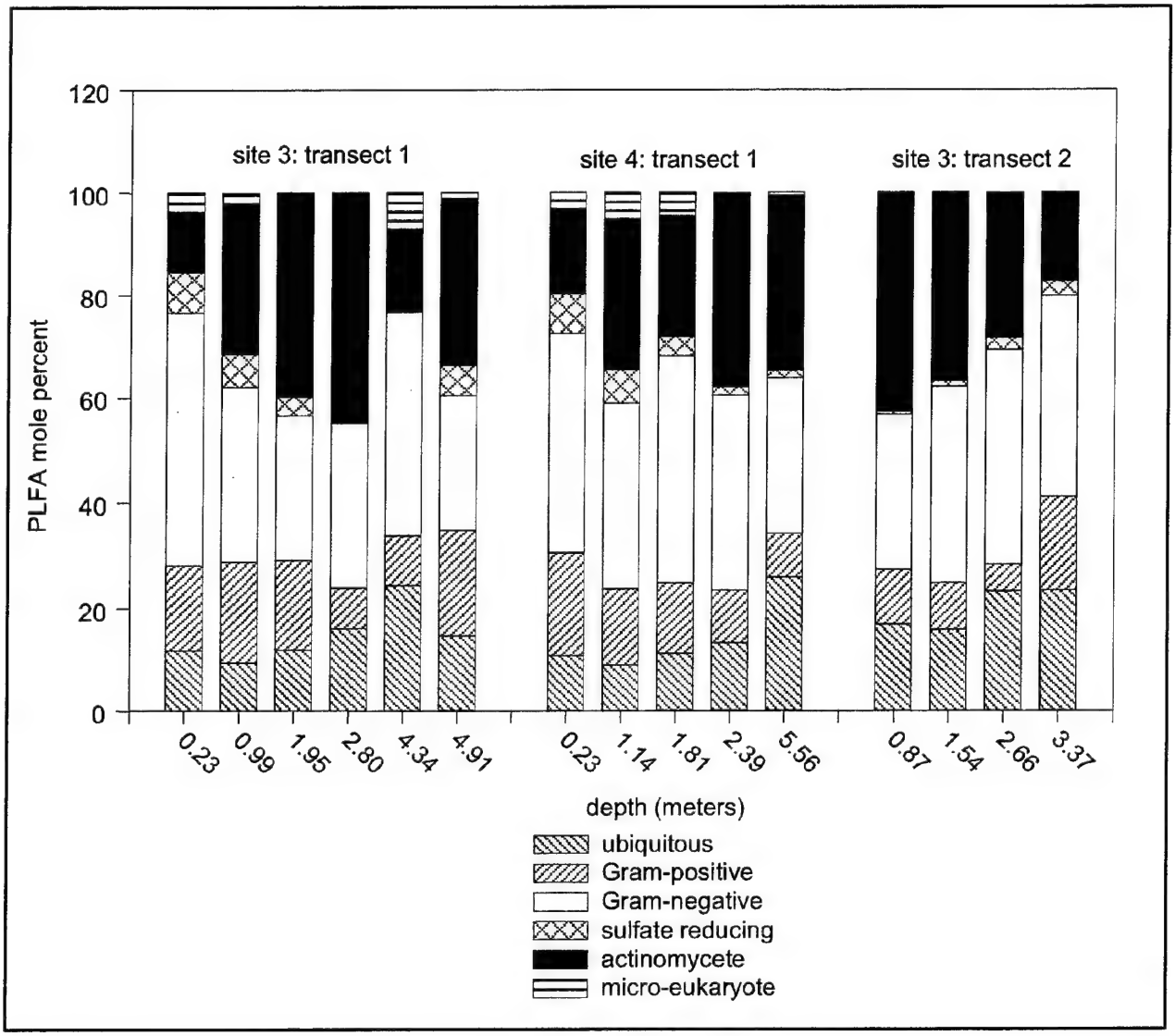


Figure 15. Microbial community composition in JAAP soils

iron-reducing bacterial abundance. In addition, the gene probes and the membrane lipid biomarkers were correlated. The nucleic acid probes showed a broader number and higher frequency of positive identifications at the JAAP site than at LAAP. This result correlates with mineralization and membrane lipid biomarker results showing greater activities and biomass at JAAP. Nucleic acid measurements also demonstrated a greater frequency of reductive or anaerobic gene probe presence at the JAAP site, which may be related to the presence of obligate anaerobes as indicated with the membrane lipid biomarker analysis.

The presence of a catabolic potential, microbial biomass, and a community composition suited to the degradation of explosives was evident at the JAAP site. The biomarker analyses also indicated that ex situ rates of TNT metabolism could be directly related to characteristics of the in situ microflora.

Table 12
Spearman Rank Order Correlations for Mineralization Rates, Contaminant Concentrations, Geochemistry, and Microbial Biomarkers at JAAP

Mineralization Rates			Contaminant Conc.			Geochemistry					Genetic Biomarkers					Membrane Lipid Biomarkers				
ACErate	RDXrate	TNTrate	TNT	2ADNT	4ADNT	% SAND	% SILT	% CLAY	pH	TOC	Nred	BIPHANA	R220-225	C23O	C23O 125 bp	Biomass	gm (+)	gm (-)	SRB/IRB	LT-stress
ACErate	0.08	0.13[1]	-0.17	-0.18	0.10	0.31	0.17	-0.49	-0.20	0.03	0.15	0.28	0.10	0.50	0.37	0.17	0.51	0.24	0.29	-0.13
	RDXrate	0.27	0.45	0.62	0.36	0.13	-0.27	0.08	0.08	-0.13	-0.17	-0.13	0.36	0.06	-0.04	-0.28	-0.05	0.24	-0.09	-0.41
		TNTrate	0.38	0.28	0.31	-0.47	0.52	0.06	-0.56	-0.52	0.48	0.54	0.31	0.50	0.52	0.58	0.33	0.42	0.60	0.64
			TNT	0.84	0.73	-0.01	0.19	0.04	0.05	-0.06	-0.30	-0.17	0.73	0.34	0.47	0.37	0.12	0.43	0.43	-0.13
				2ADNT	0.61	0.21	-0.06	-0.18	0.02	0.06	-0.39	-0.21	0.61	0.24	0.36	0.04	0.27	0.42	0.34	-0.19
					4ADNT	0.26	0.13	-0.21	-0.26	-0.31	-0.21	-0.12	1.00	0.54	0.68	0.37	0.12	0.43	0.43	-0.13
						%SAND	-0.56	-0.71	0.09	0.22	-0.58	-0.11	0.26	0.14	0.08	-0.14	0.19	0.18	0.01	-0.46
							%SILT	0.00	-0.56	-0.38	0.46	0.28	0.13	0.28	0.40	0.50	0.50	-0.13	0.58	0.60
								%CLAY	0.32	-0.02	0.21	-0.13	-0.21	-0.33	-0.36	-0.20	-0.67	-0.28	-0.39	-0.14
									pH	0.67	-0.51	-0.60	-0.26	-0.50	-0.45	-0.65	-0.59	-0.22	-0.79	-0.68
										TOC	-0.49	-0.38	-0.31	-0.31	-0.19	-0.47	-0.26	0.02	-0.43	-0.50
											Nred	0.55	-0.21	0.24	0.07	0.31	0.15	0.09	0.19	0.53
												BIPHANA	-0.12	0.60	0.31	0.58	0.27	0.35	0.46	0.52
													R220-225	0.54	0.68	0.37	0.12	0.43	0.43	-0.13
														C23O	0.79	0.62	0.31	0.66	0.58	0.11
															C23O 125 bp	0.59	0.36	0.50	0.59	0.23
																Biomass	0.40	0.35	0.79	0.60
																	Gram (+)	-0.10	0.75	0.45
																		Gram (-)	0.22	0.01
																			SRB/IRB	0.57
																				LT-stress

[1] Shaded cells indicate coefficients of 0.5 or greater, which are significant at a 95-percent confidence level.

Discussion—Biomarkers as Tools for the Assessment of Natural Attenuation

Degradation potential

In situ rates for explosive degradation/transformation were extrapolated from laboratory radiorespirometry. In general, soil samples from the LAAP site supported mineralization of acetate at discrete depths within the soil profiles. Both RDX and TNT mineralization activities were present, but only at very low levels. In many cases, the $^{14}CO_2$ was bound within the soil as carbonates. While the numbers of microorganisms present at the LAAP site were low, the microbes were responsive to the presence of readily available organic carbon (acetate). These microorganisms possessed some genes indicative of explosives-mineralizing potential. However, only slow mineralization of very low levels of RDX or TNT in response to the addition of radiolabeled compounds was observed. At the JAAP site, microorganisms able to rapidly mineralize acetate were prevalent and distributed so that the highest levels were found near the surface (activity declined with depth). JAAP microorganisms demonstrated moderate rates and extents of TNT mineralization, even when TNT was not detected in the sample or was detected at low levels.

Genetic potential

Evidence of an in situ explosive catabolic potential was derived from genetic testing at both sites. The distribution and frequency of the designed probes were slightly different at each site. The gene probes were able to identify key steps in processes of catabolic pathways vital to explosives degradation. Evidence supported both reductive and oxidative pathways. TNT mineralization rates correlated positively with genes coding for several enzymes considered important to TNT degradation. Rates of TNT mineralization also correlated positively with microbial biomass, the relative abundance of sulfate- and iron-reducing bacteria, and was tied to lipid biomarkers, which are indicators of long-term environmental stress within the gram-negative bacterial population. Genetic biomarkers consisting of genes coding for several key enzyme systems were present at both sites, although the specific kinds of these enzymes differed between sites. Based on the conceptual pathway for TNT degradation (Rieger and Knackmuss 1995), nitroreductase and biphenyl dioxygenase (245 bp)-related genes indicative of microaerophilic to anaerobic conditions were present at both sites. While the literature contains proposed pathways for RDX degradation occurring under anaerobic, oxygen-depleted, and nitrogen-limiting aerobic conditions (McCormick, Cornell, and Kaplan 1981; Kitts, Cunningham, and Unkefer 1994; Binks, Nicklin, and Bruce 1995), the enzymes required have not been established. These pathways may require enzymes similar to atrazine reductase and nitroreductase, both of which were found at LAAP; but only atrazine reductase was found at JAAP. The extent of mineralization was also greater at JAAP.

Genes reflective of aerobic conditions were also present at both sites. Moreover, when the level of biphenyl dioxygenase was examined at JAAP, the

abundance of this gene was related to the concentrations of TNT, 4ADNT, and 2ADNT. The presence of this enzyme is a result of both sites having been exposed to TNT. However, extensive mineralization of TNT was only found at JAAP. Environmental factors regulating activity at JAAP may, therefore, be more conducive to explosives degradation.

Microbial biomass, community structure and function

The presence of a viable microbial biomass with the capacity to mineralize explosives (ex situ) was established using lipid biomarkers and radiorespirometry. Application of lipid biomarkers provided an accurate and reproducible estimate of the viable subsurface microflora at each site. The JAAP site supported a subsurface microflora an order of magnitude larger than that observed at LAAP. The subsurface microflora at JAAP was also capable of mineralizing radiolabeled TNT at rates 5 to 10 times faster than that found in the LAAP subsurface soils. Moreover, this technique identified differences in community structure attributable to the presence of contamination. The ability to quantify differences in subsurface microbial communities (those taken in close areal proximity with the only difference being the presence or absence of contamination) provided a measurement for evaluating progress towards natural attenuation at LAAP. The purpose of this portion of the work was to determine whether or not a given biomass could be directly related to RDX or TNT mineralization potentials. A microbial community "signature," as described by PLFA analysis, was developed for two to three situations within each of the two sites. In both cases, a significant correlation between microbial biomass and TNT mineralization rates was observed. In LAAP soils, the correlation was weakly negative, indicating that as TNT mineralization increased, biomass decreased (Table 9). This means that TNT had a negative effect on (tended to diminish the growth of or to destroy) the microbial biomass at LAAP. However, the limited biomass was able to mineralize TNT. By contrast, for JAAP soils the correlation between TNT mineralization and biomass was strongly positive (Tables 13 and 14). These results are likely a function of the significantly higher explosives concentrations at LAAP.

The composition and physiological status of the in situ microbial communities were also determined with lipid biomarkers. In addition to the considerable differences in location, explosives concentrations, nutrients, organic matter levels, pH, and other site-specific parameters between LAAP and JAAP, the microbial communities were also very different. Lipid community signatures related to TNT concentration and mineralization in both LAAP- and JAAP-contaminated soils were identified. Relationships to contaminant concentrations were more pronounced at LAAP, since the contamination was greater and more widely distributed. Specific traits of the LAAP in situ microbial community were directly related to contaminant exposure—i.e., increased evidence of physiological stress and the relative percentages of gram-positive bacterial biomarkers in soils collected from the same mean depth (Community 1 versus Community 3 in Table 8). The increased percentage of gram-positive bacteria with TNT contamination represents a positive community attribute, assuming *Clostridium* is included. The impact of TNT and RDX contamination on

Table 13
Summary of Spearman Correlations Comparing LAAP and JAAP Mineralization with Explosive Concentrations, Geochemistry, and Biomarkers

	Mineralization Rates					
	LAAP			JAAP		
Variable	Acetate	TNT	RDX	Acetate	TNT	RDX
Explosive Concentrations						
TNT		X[1]				
TNB		X				
2ADNT						X
RDX		X				
HMX		X				
Geochemistry						
pH					X(-)[2]	
%Silt		X(-)			X	
TOC					X(-)	
Genetic Biomarkers						
NAD(P)H Flavin Nitroreductase	X(-)				X	
Catechol-2,3-dioxygenase	X(-)	X		X	X	
Catechol 2,3-dioxygenase 125 bp					X	
Membrane Lipid Biomarkers						
Viable biomass			X(-)		X	
Gram-positive				X		
Obligate anaerobe					X	
Actinomycete	X				X	
Long-term stress					X	
Short-term stress	X					

[1] X indicates significant positive correlation ($p < 0.05$).
[2] X(-) indicates significant negative correlation ($p < 0.05$).

microbial community composition is undoubtedly a process of selection, not of stimulation, since total viable biomass decreased with increasing TNT and RDX concentrations. The selection for a specific community of bacteria, particularly one able to transform/mineralize the contamination, is conducive to the natural attenuation of the site. The lipid measurement showing increased physiological stress also represents a positive community trait, since this property shows a response, other than death, by the native microorganisms to the presence of contaminants.

Table 14
Multiple Regression Analysis of the Variables at LAAP and JAAP Against TNT Mineralization Rates[1]

LAAP		JAAP	
Variable	**Beta[2]**	**Variable**	**Beta**
[RDX]	1.58	Total viable biomass	0.78
[HMX]	-0.63	pH	-0.52
Total phosphate	-0.46	[TNT]	-0.41
Eukaryote %	0.25	Anaer/aer or mic/arth	0.20
Total organic carbon	-0.22	Gram-negative %	0.14
Total viable biomass	-0.03	Dissim. sulfite reductase 320 bp	-0.04
Catechol-2,3-dioxygenase 195 bp	0.02		

[1] Significant variables at 95-percent level of confidence ($p < 0.05$).
[2] Beta coefficient is the multiple regression R value.

Performance at the two sites

For LAAP, most of the significant correlations obtained for genetic and membrane lipid biomarkers were related to acetate mineralization. No strong correlations were found with either RDX or TNT mineralization. The soil at LAAP is nutrient poor and has little/no readily available organic matter to support cometabolic destruction of RDX or TNT, even though high levels of both explosives were present in different areas of this site. In contrast, the JAAP site is rich in organic carbon, but nitroaromatic concentrations were lower, indicating that the site is able to support cometabolic destruction of TNT and RDX. Many significant correlations between TNT mineralization and nucleic acid or lipid biomarkers were observed for the JAAP site.

Results from a multiple regression analysis comparing biomarker, geochemistry, and contamination against TNT mineralization rates at both sites is provided in Table 14. At least one variable from each of the four groups examined (nucleic acid biomarkers, lipid membrane biomarkers, explosives concentrations, and geochemical parameters) was found to be significant at each site. Although this test did not satisfy normality assumptions and suffered from a small sample size, the most significant indictors of TNT mineralization (i.e., natural attenuation) at each site were (a) total viable biomass, (b) membrane lipid biomarkers for eukaryotes (LAAP) and gram-negative bacteria (JAAP) and for anaerobic conditions (JAAP), (c) genes associated with aerobic biodegradation at LAAP (catechol-2,3-dioxygenase) and anaerobic biodegradation at JAAP (dsrB), (d) the geochemical parameters of total organic carbon and total phosphate (LAAP) and pH (JAAP), and (e) the concentration of at least one of the explosives (TNT, RDX, or HMX). In addition, catechol-2,3-dioxygenase genes, present at both LAAP and JAAP, may prove to be a useful biomarker.

The two biomarker tools supplied complementary evidence for demonstrating the occurrence of natural attenuation at each site. Membrane lipid biomarkers described a viable biomass and effects of the explosives contamination on microbial community composition; whereas, the nucleic acid biomarkers

described a mineralization capability. The combined nucleic acid and membrane lipid biomarker techniques provided a comprehensive evaluation of the attenuation mechanisms prevalent at each site.

Conclusions

Integration of results from radiorespirometry, genetic, and membrane lipid biomarker techniques was used to evaluate the ability of indigenous microorganisms to degrade explosives. For the two sites, membrane lipid biomarker technology provided estimates of viable cell abundance. By identifying the amount and nature of the in situ viable microflora, in relation to nitroaromatic contamination, a direct link was established between the rates of contaminant mineralization observed in the radiorespirometry flasks and the existing microbial populations. The genetic biomarkers provided the necessary evidence of a genetic capability for natural attenuation at each site. Biomarkers at both sites provided positive evidence that microbial transformation/mineralization processes play a substantial role in explosives attenuation at these sites.

Rates of TNT and RDX mineralization were very low in LAAP soils, and few significant correlations were found when these rates were compared with geochemical parameters or biomarkers. However, TNT mineralization rate did correlate positively with explosives concentration and a catechol oxygenase gene. The TNT mineralization rate for JAAP correlated positively with both nucleic acid and lipid biomarker parameters.

Aerobic degradation of TNT in LAAP soils was indicated by the presence of two catechol oxygenases gene probes. Therefore, the microbial populations contained some, if not all, of the necessary genes for explosives mineralization. The occurrence of the denitrification enzyme in TNT-contaminated JAAP subsurface soils indicated that the mechanism necessary for microbial reduction of TNT was present, while other observed genes support both anaerobic and aerobic metabolism of the aromatic rings. The nucleic acid investigation provided evidence that catabolic genes are reliable biomarkers for contaminant presence and mineralization potential.

Low explosives-mineralization rates and lipid biomarker results together suggest that only a segment of the native microbial community was able to mineralize nitroaromatic contaminants at LAAP. At JAAP, mineralization rates were considerably higher. Several nucleic acid probes had positive correlations with mineralization rates, but the most significant positive relationships found in multiple regression analyses against TNT mineralization rates were with lipid membrane biomarker measures of microbial biomass, estimates of gram-negative and sulfate- and iron-reducing bacterial abundance, as well as the anaerobic enzyme dissimilatory sulfite reductase. This suggests that a substantial portion of the microbial community was able to mineralize nitroaromatic contaminants at JAAP and provides a strong tie to a role for anaerobic or anaerobic/aerobic switching conditions in regulating microbial degradation of this explosive.

Factors identified as exerting the most significant influence on natural attenuation processes at both sites included the following: TNT and TNT transformation product concentrations, soil texture, microbial biomass and community structure, and genes for several specific enzymes (sulfite reductase, nitroreductase biphenyl dioxygenase, and catechol-2,3-dioxygenase). This information can be used in extrapolation of results from laboratory data to field conditions. Field-generated contaminant data combined with biomarker data will permit predictions of the rate and extent of explosives transformation and mineralization. A comprehensive view of how microbial community characteristics relate to RDX and TNT mineralization can be developed for the future analyses of additional sites.

The data developed at the LAAP and JAAP sites represent a single snapshot of the catabolic potential at each location. Periodic reexamination can verify that short-term observations based on samples obtained over a few days will hold true over the long term (decades). Repeated assessments of biomarkers can be utilized as a monitoring tool to supplement and support chemical groundwater monitoring. Biomarker data can be synthesized to produce a dynamic picture of degradation over time that can be directly related to ex situ measurements of TNT mineralization rates, enhancing the value of biomarkers as tools. The techniques developed at LAAP were validated in JAAP soils. The biomarker tools applied to the site collectively support the potential for active in situ transformation/degradation of nitroaromatic contaminants. Integration of the various biomarker results provide a direct link between the ex situ measure of rates of mineralization (radiorespirometry) and the in situ microbial communities.

The advent of biochemical molecular tools, including lipid and nucleic acid biomarkers, has vastly improved the ability to assess the effectiveness of natural attenuation. Biomarkers are able to demonstrate microbial destruction of the contaminant in field samples. In addition, biomarkers provide the information required to predict the rate and extent of explosives degradation and transformation. The success of biologically mediated natural attenuation depends on site characteristics, the composition and abundance of the subsurface viable biomass, and the genetic capability to metabolize onsite contamination. The capability of biomarkers to measure the effectiveness of natural attenuation for degradation of explosives at LAAP and JAAP has been demonstrated.

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Glossary

The following terms specific for nucleic acid biomarkers are defined in terms of their composition and function.

Backtranslated multiple sequence alignments - The basic structural unit of proteins is a string of amino acids. This amino acid string is specified by a string of nucleotides in a gene. The DNA sequences of a gene can be determined (backtranslated) from the amino acid string or an enzyme. By aligning the backtranslation (DNA sequence) of related enzymes, a primer can be developed that will recognize a number of different but related genes.

Catabolic gene - A region of DNA that specifies (codes for) an enzyme involved in the degradation of a compound.

DNA - Nucleic acids that specify the formation of functional (enzymes) and structural proteins. DNA is present when no enzymes are currently expressed.

Functional genes - Genes that encode enzymes that catalyze a specific function, such as degradation of a contaminant.

Genetic standards - A mixture of genes or bacterial DNA containing genes that are to be detected. This material is used as a positive control.

Nucleic acids - The genetic coding materials used by living organisms. These include deoxyribonucleic acid (DNA) and ribonucleic acids (RNA).

Polymerase chain reaction (PCR) - An enzyme-based amplification (multiplication) procedure. It uses a heat stable DNA polymerase, an enzyme that makes copies of DNA. The reaction begins by separation of the double-stranded DNA by heat (denaturation). The temperature is reduced so that primers can bind to the DNA (annealing). The temperature is then raised to permit DNA synthesis. After synthesis, the double-stranded fragments are again heat-denatured, primers annealed, and new DNA synthesized. This process is repeated 25 to 40 times, resulting in an exponential amplification of a target gene. PCR produces enough gene fragments so that even low amounts of a gene can be detected.

Primers - Short (15 to 40 bp) pieces of synthetic DNA complementary to specific regions of a target gene that will be amplified by PCR. Primers are necessary for DNA polymerase to initiate DNA copying. Two primers are required for PCR. Each primer is paired with another primer, one for each strand of DNA. The pairing results in a bracketing of a region of DNA that can then be copied.

RNA - Nucleic acids that serve as the intermediate between genes (DNA) and the proteins. RNA is principally present only when proteins are actively being produced.

Target gene - A region of DNA that specifies an enzyme that is to be detected by PCR (See below).

Appendix
DNA Extraction

Method for DNA extraction (Zhou, Bruns, and Tiedje 1996)

Ten grams of soil is suspended into 13.5 mL DNA Extraction Buffer (100 mM Tris Hcl, pH 8.0; 100 mM EDTA, pH 8.0; 100 mM sodium phosphate, pH 8.0; 1.5 M NaCl, 1 percent w/v CTAB). One hundred microliters of 10-mg/mL proteinase K (Sigma Company, St. Louis, MO) (dissolved into DNA extraction buffer) and 135 mg (1 percent w/v) polyvinylpolypyrrolidone (Sigma). Tubes are incubated in a rotary hybridization oven for 30 min at 37 °C. Sample tubes are removed, 1.5 mL 20-percent sodium dodecyl sulfate is added to each sample and tubes returned to the rotary hybridization oven to incubate for 2 hr at 65 °C. After incubation, debris is removed by centrifugation at 6,000 RCF for 10 min. Supernatants are sieved through sterile cheesecloth to fresh 50-mL tubes. Soil debris pellets are reextracted twice more with 4.5 mL DNA extraction buffer, 0.5 mL 20 percent SDS and incubation at 65 °C for 10 min. Supernatants are filtered through cheesecloth and pooled. The pooled supernatants are extracted with an equal volume of chloroform:isoamyl alcohol (24:1). After centrifugation at 13,800 RCF for 10 min, the aqueous phase is removed to fresh tubes. Sixty microliters of 5-mg/mL mussel shell glycogen (Ambion, Austin, TX) was added to the extracted DNA solution for a final concentration of 10-μg/mL as a coprecipitant to enhance recovery of DNA from dilute solutions. To precipitate DNA from solution, 0.6 × volumes of isopropanol and 2 mL 7.5 M ammonium acetate are added. Samples are precipitated at room temperature for at least 1 hr. DNA is recovered by centrifugation at 13,800 RCF for 40 min. DNA pellets are washed with 70-percent ethanol, air dried, and resuspended into 20 µL distilled water.

The thermal profile schedule for LAAP PCR is as follows:

a. Twelve minutes of denaturation at 95 °C.

b. Two cycles of 20-sec denaturation at 94 °C.

c. One minute of annealing at 55 °C.

d. Three minutes of extension at 72 °C.

e. Two cycles of 20 sec denaturation at 94 °C.

f. One minute of annealing at 57 °C.

g. Three minutes of extension at 72 °C.

h. Thirty cycles of 20 sec denaturation at 94 °C.

i. One minute of annealing at 59 °C.

j. Three minutes of extension at 72 °C.

k. Thirty cycles of 20 sec denaturation at 94 °C.

l. One minute of annealing at 61 °C.

m. Three minutes of extension at 72 °C.

The thermal profile schedule for JAAP PCR is as follows:

a. Fifteen minutes of denaturation at 95 °C.

b. Thirty-five cycles of the following:

(1) Twenty second denaturation at 94 °C.

(2) One minute of annealing at 60 °C.

(3) Four minute of extension at 72 °C.

c. Ten minutes of final extension at 72 °C.

5 Numerical Modeling

Introduction

Numerical modeling can be used to integrate site hydrogeology and contaminant distribution with results from laboratory testing and biomarker investigations to predict the effectiveness of natural attenuation in regulating contaminant transformation and transport. The combined effect of advection, adsorption, and degradation on the persistence of explosives can be evaluated. At the LAAP site, explosives may be sorbed by soil particles, immobilized by chemical interactions with soil components, and degraded by microorganisms. These processes may vary spatially. The transport of chemicals can occur by percolation of water and may be retarded or delayed compared with the fluid flow. Biochemical, chemical, and physical processes collectively act to modify contaminant concentration and distribution over time and space. Field measurements provide a snapshot of the site geochemistry and microbiology at a specific time. Chemical, physical, and biological data from a site can be integrated using numerical models into a framework that supports natural attenuation of explosives.

Numerical modeling was applied to the LAAP site using information derived from site monitoring, site-capacity testing, and biomarker analyses. The objectives of the numerical modeling effort were as follows: (a) to provide visualization of contaminant distribution at the site, (b) to evaluate the dominant factors affecting natural attenuation at the site, and (c) to predict long-term contaminant migration and transformation.

Groundwater Modeling System (GMS)

The Department of Defense Groundwater Modeling System (GMS) (1996) Version 2.0 is a comprehensive computer graphical system, which includes modeling tools to facilitate site characterization, model conceptualization, mesh and grid generation, geostatistical computations, and postprocessing. GMS is a state-of-the-art graphical computer interface that is linked with groundwater transport and water quality models to predict the fate and transport of contaminants at a site.

To visualize and model the distribution of explosives at the LAAP site, GMS with its subsurface flow and transport model, FEMWATER (Lin et al. 1997), was

applied. Several geostatistical (interpolation/extrapolation) numerical tools are integrated into GMS for this purpose. In the three-dimensional (3-D) space, these include inverse distance weighted, natural neighbor, and kriging. Each of these approaches has its own merits. The reader is referred to GMS user's manual for detailed descriptions of these options (GMS 1996).

Conceptual Model

The first step in numerical modeling is to develop a conceptual model that describes essential components of natural phenomena and hydrogeological conditions in a simplified form. The conceptual model was based on LAAP site geological and chemical data that incorporated information from the site borehole geology, hydraulic conductivity, and flow boundary conditions.

Hydraulic conductivity, porosity, recharge rates, and contaminant concentrations vary spatially over the site. Full characterization of the heterogeneity of the aquifer is not possible because of limitations in hydraulic conductivity measurements and associated errors and uncertainties. In addition, definitive information about boundary conditions and recharge rates is usually unavailable because of the complexity of the geology of the aquifer and lack of reliable means to measure or estimate fluxes at boundaries or recharge rates and their distribution. For this site, discrete hydraulic conductivity data were interpolated/extrapolated to estimate properties at intermediate points of the numerical mesh. A combination of GMS geostatistical tools was used to develop a numerical representation of the site.

The modeling domain at the ground surface of the LAAP site included the former lagoon area and proximal monitoring wells (Figure 1). Four stratigraphic units were identified at LAAP based on lithologic data. The units were a vadose zone (unsaturated soil), an Upper Terrace aquifer, a semiconfined layer, and a Lower Terrace aquifer. The vadose zone and the Upper Terrace aquifer form a shallow unconfined aquifer. The terrace deposits are composed of alternating beds of mixed sands and clay. The values of hydraulic conductivity for each layer and node of the 3-D modeling mesh (Figure 2) were estimated from the data given in Table 1 using GMS geostatistical tools.

The original and average distributions of subsurface geologic materials were derived from cone penetrometer (CPT) data and interpolated using GMS. Figure 3 illustrates the site subsurface heterogeneity. The material distribution as shown in Figure 4 formed the basis for definition of subsurface layers for modeling purposes. The measured hydraulic conductivities of the three cap material samples were approximately 10^{-6} cm s^{-1}; therefore, infiltration through the cap was considered negligible.

The numerical model requires flow information at the boundaries of the modeling domain. The water-level elevations measured at the monitoring wells were used to estimate transient flow boundary conditions.

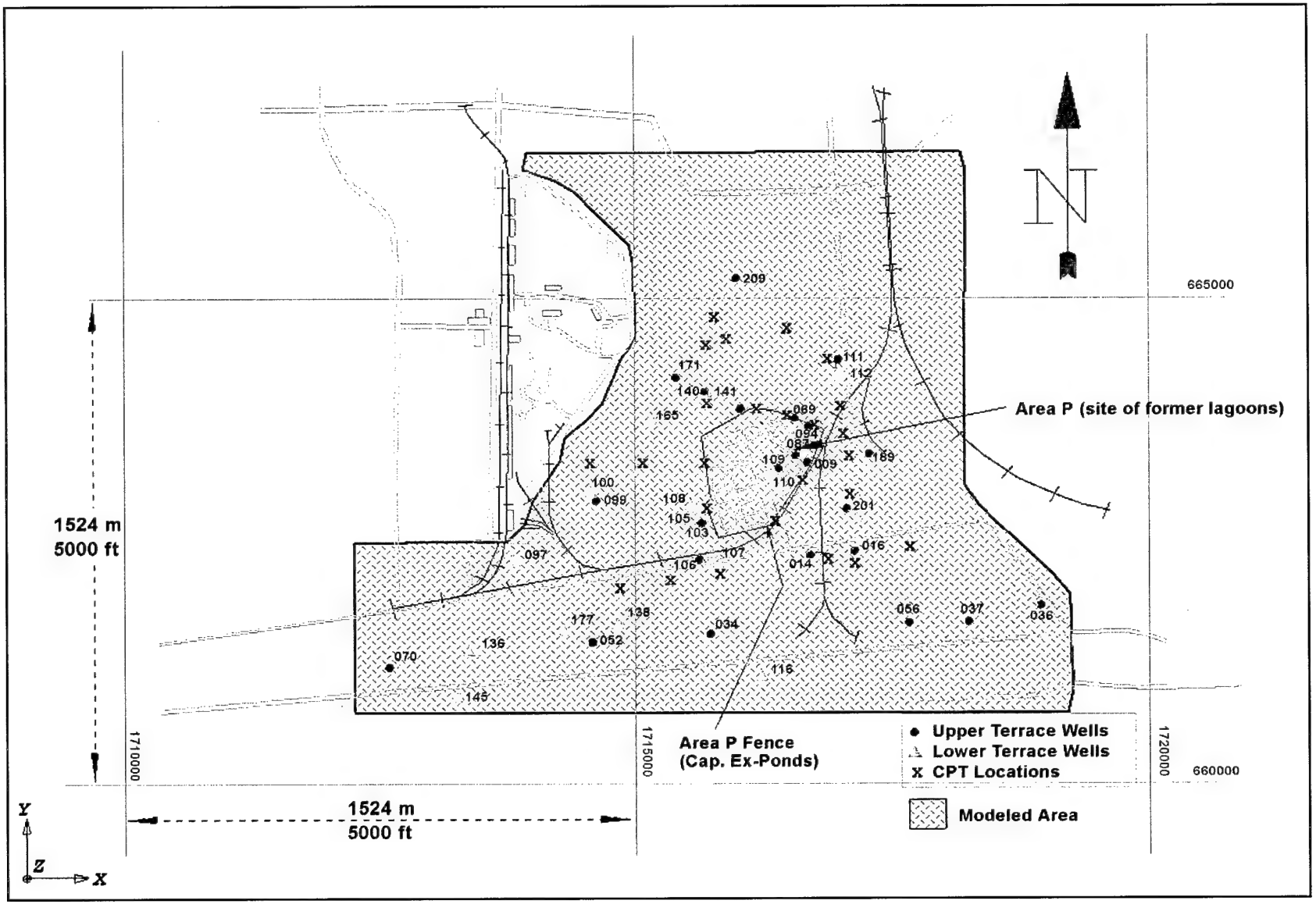

Figure 1. Modeled area at LAAP included Area P (site of former lagoons) and surrounding groundwater monitoring wells

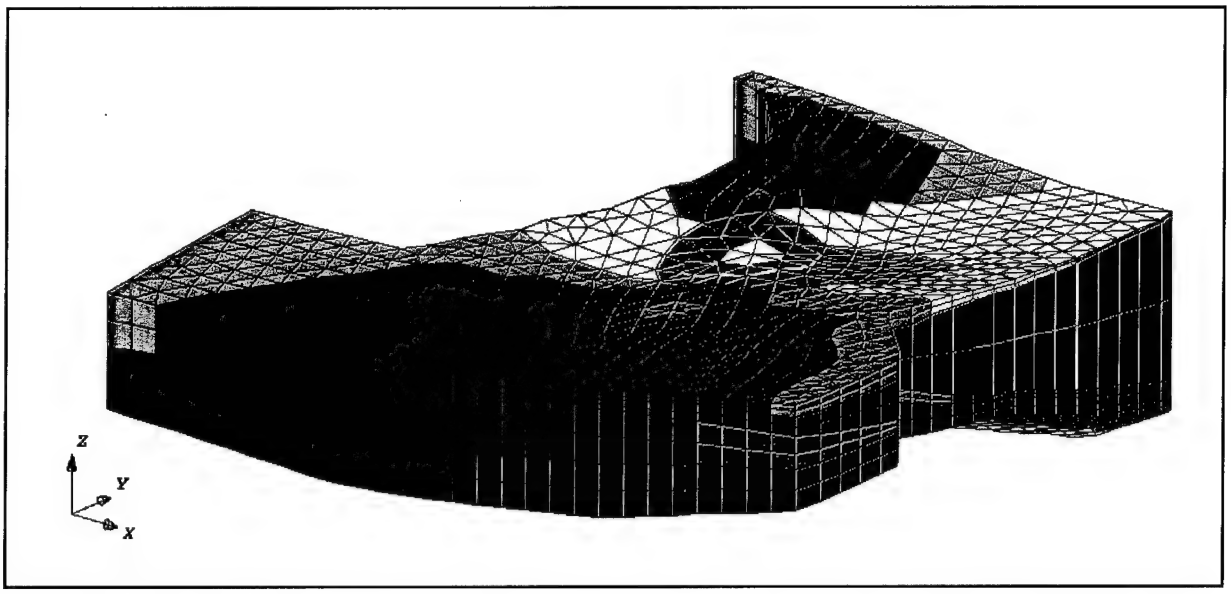

Figure 2. Site heterogeneity illustrated by three-dimensional mesh system used in FEMWATER (colors representing different materials or hydraulic conductivity distribution)

The source of flow recharge at Area P was assumed to be solely from rainfall. The precipitation data were collected at Minden, LA (Figure 5). Median values ranged from 6 to 12 cm per month. The rainfall data were used to estimate average infiltration rate for the site.

Code Description

The model selected for this study, FEMWATER (Lin et al. 1997), is a 3-D finite element numerical code, which may be used to simulate flow and mass transport through saturated-unsaturated media. FEMWATER is an enhanced version of two models, 3DFEMWATER (flow) and 3DLEWASTE (transport). FEMWATER is integrated into GMS (1996). The flow equations in FEMWATER are based on the continuity and Darcy flow equations. The model application is limited by the assumptions applied to these equations relating to laminar flow conditions, incompressible fluid and solid phases, and constant fluid viscosity and density. The Darcy formula (or law) defines the water flow rate (Q;L^3/T) in a cylinder filled with sand with cross-sectional area A (L^2) as:

$$Q = K A \frac{(h_1 - h_2)}{XL} \tag{1}$$

where

K = hydraulic conductivity (L/T)

h_1 = hydraulic head at upstream (Point 1)

Table 1
Hydraulic Conductivity at Cone Penetrometer (CPT) Locations

CPT Location	Surface Elevation, m	Depth to CPT Point m	X, m	y, m	z, m	Conductivity (m/day)[1]
2-1	66.127274	18.898	522937.1158	208275.66	47.229674	9.708
2-3	65.73835	5.547	522752.6871	208277.31	60.19099	0.024
2-5	66.672257	12.161	522593.9155	208277.33	54.51067	0.088
2-5	66.672257	7.498	522593.9155	208277.33	59.17411	0.003
4-4	70.69013	18.288	522944.4148	208644.91	52.40214	6.215
4-4	70.69013	6.187	522944.4148	208644.91	64.5027	0.116
4-4	70.69013	15.453	522944.4148	208644.91	55.23678	1.317
4-4	70.69013	20.239	522944.4148	208644.91	50.45142	11.387
4-4	70.69013	4.572	522944.4148	208644.91	66.11814	2.917
4-5	65.904161	15.149	522944.8665	208132.76	50.755637	0.003
4-5	65.904161	20.452	522944.8665	208132.76	45.452117	9.309
4-5	65.904161	15.241	522944.8665	208132.76	50.664197	2.381
4-5	65.904161	14.204	522944.8665	208132.76	51.700517	3.099
5-1	68.076166	6.858	523387.6479	207960.94	61.218172	0.046
5-1	68.076166	16.337	523387.6479	207960.94	51.738892	0.003
6-1	68.835118	11.582	523266.4917	208394.07	57.252763	1.615
6-1	68.835118	7.437	523266.4917	208394.07	61.398043	1.189
6-1	68.835118	14.905	523266.4917	208394.07	53.930443	3.018
6-2	68.676317	10.363	523183.7218	208426.06	58.312964	9.205
6-2	68.676317	12.253	523183.7218	208426.06	56.423204	3.185
6-2	68.676317	17.496	523183.7218	208426.06	51.180644	1.524
6-3	68.285258	6.248	523092.1714	208445.32	62.03677	3.169
7-1	67.580256	16.002	523149.2257	208092.33	51.578177	0.091
7-1	67.580256	7.041	523149.2257	208092.33	60.539297	0.046
7-1	67.580256	4.999	523149.2257	208092.33	62.581457	0.061
7-2	68.350181	17.739	523231.2175	208221.28	50.610809	0.091
7-2	68.350181	10.638	523231.2175	208221.28	57.712649	3.139

[1] Based on the standard sieve analysis and U.S. Army Corps of Engineers Unified Soil Classification System (1960).

h_2 = hydraulic head at downstream of cylinder (Point 2)

XL (L) = length of cylinder

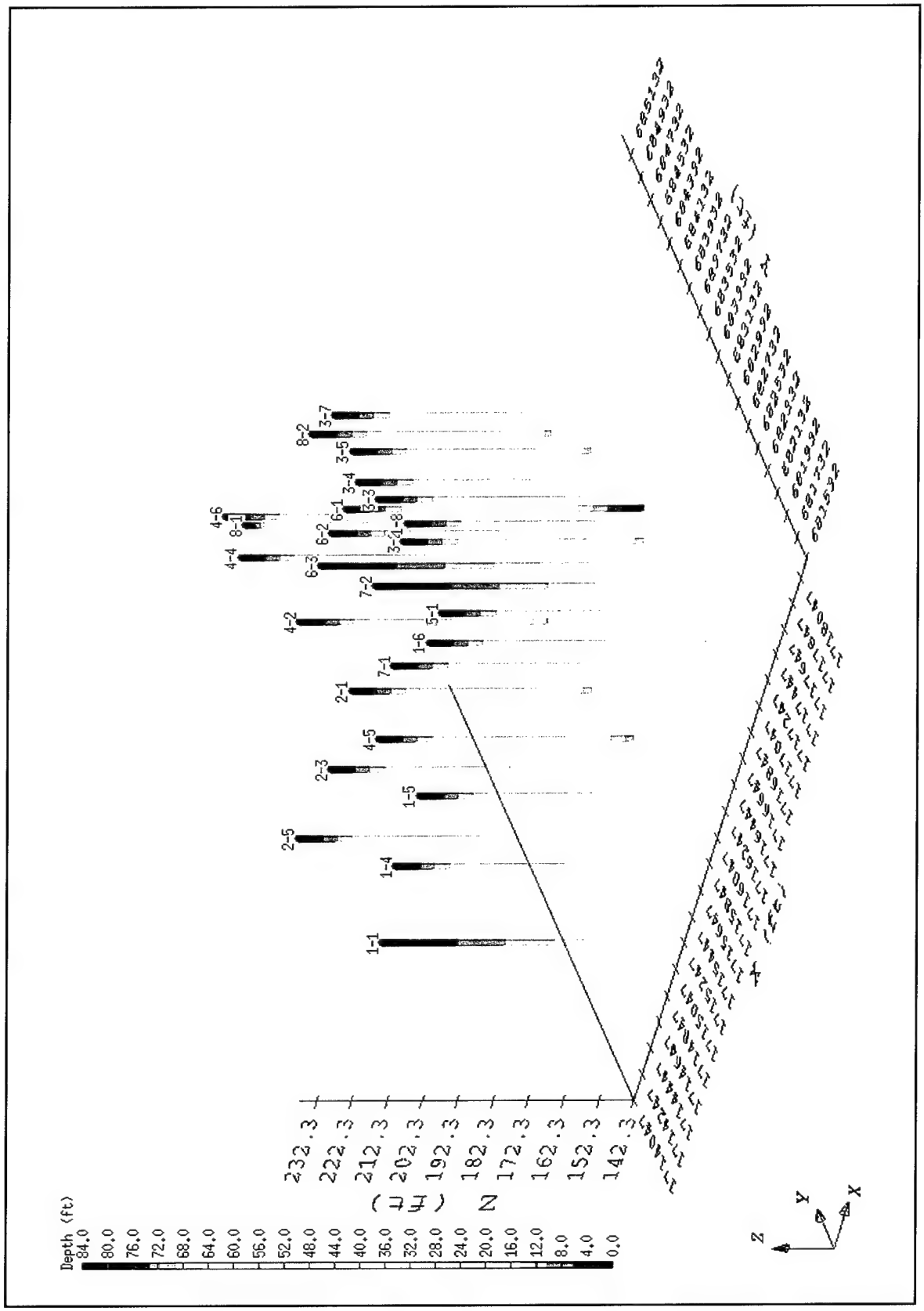

Figure 3. Original stratigraphy distribution based on CPT data (depth of different materials)

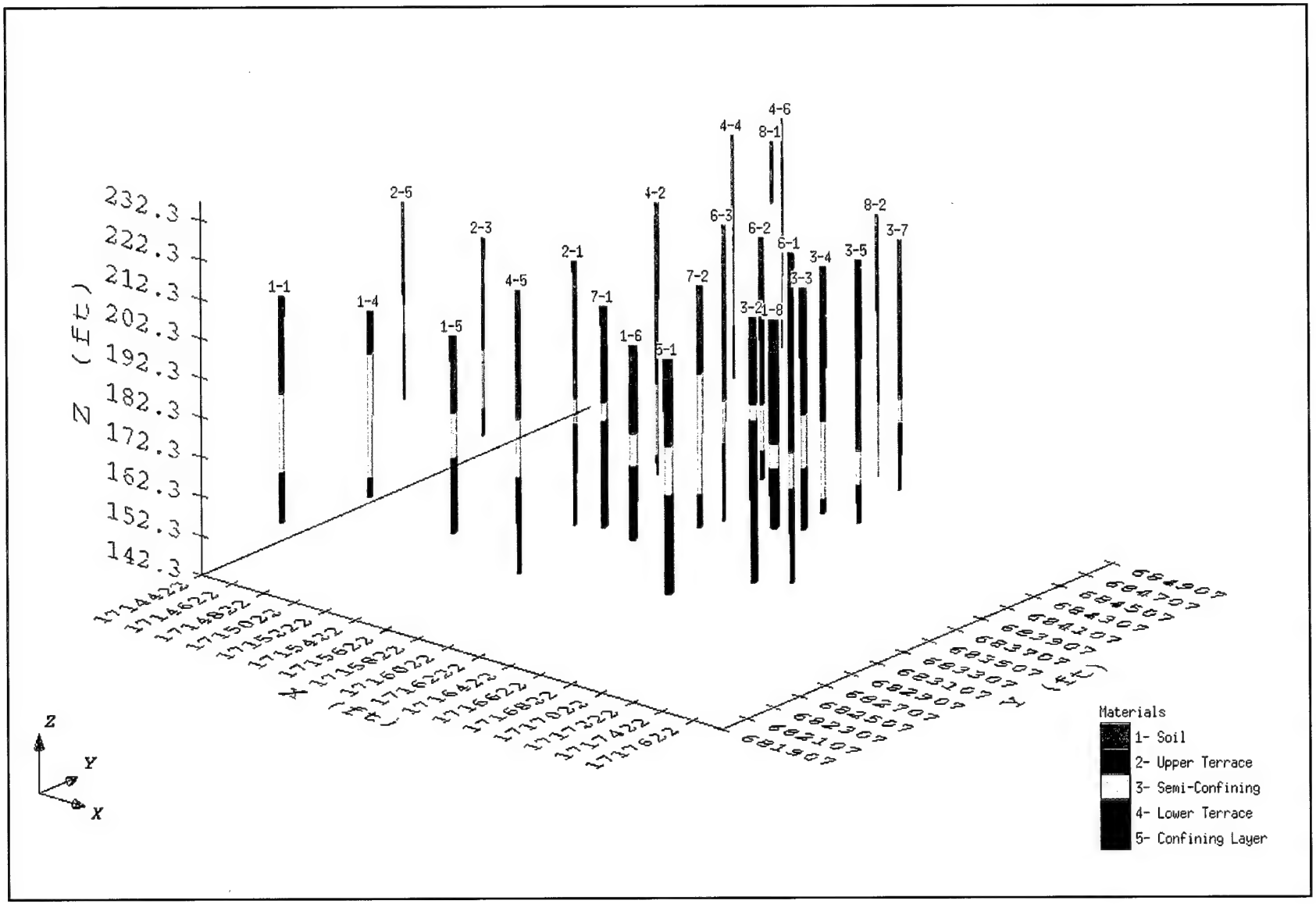

Figure 4. Stratigraphy averaged into five layers at each CPT location (Confining (impervious) layer served as a boundary and was not included in model)

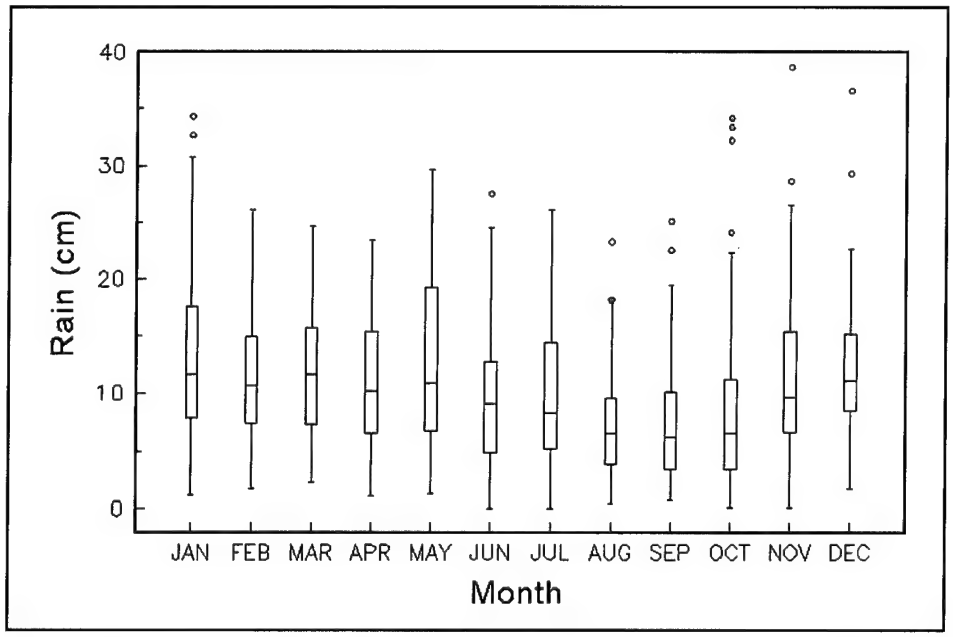

Figure 5. Statistical representation of rainfall data measured at Minden, LA, during 1931-1992 (Horizontal line in each box represents median amount of rainfall per month; limits of box represent 95-percent confidence interval for each month over time period from 1931 to 1992; vertical bars represent range of values)

FEMWATER simulates the primary processes affecting dissolved-phase contaminant distributions in groundwater including advection, dispersion, sorption, and decay caused by chemical reactions and biological transformation. In most groundwater mass transport models, biodegradation in groundwater systems is assumed to follow zero- or first-order decay processes (Kosson, Agnihotri, and Ahlert 1995). In FEMWATER, microbial and physical/chemical-removal mechanisms are collectively represented using an apparent first-order decay rate coefficient.

FEMWATER requires three data sets containing soil parameters for unsaturated-saturated materials. A summary of input and output files used with GMS/FEMWATER is given in Tables 2 and 3, respectively. In unsaturated flow domains, the hydraulic conductivity K varies with the soil potential head h, which is also a function of the volumetric soil moisture content θ (Equations 2 through 4).

Site-specific information for moisture content, relative hydraulic conductivity, and water content are needed to link the input files with the pressure-head distribution. Two options are available in GMS: the user can select an automatic generation of these parameters based on van Guenthen (1980) or input these parameters manually using other available empirical equations such as the one developed by Brooks and Corey (1964). For the LAAP application described here, Brooks-Corey formulations were selected to allow for representation of each soil type.

Table 2 FEMWATER/GMS Input Files	
File Name	**Description**
Super File	Text file containing a list of all of the input and output files used in FEMWATER simulation.
Geometry File	Text file containing the data describing the finite element mesh, i.e., node coordinates and element topology.
Mode File	Text file containing analysis parameters and options, material properties, boundary conditions, and initial condition options.
Initial Condition File	Text or binary files containing concentration, pressure head, velocity, moisture content, initial conditions.
Flow File	Text or binary files containing a previously computed flow solution (pressure head and velocity) that are used to define a 3-D flow field for transport-only simulation.

Table 3 FEMWATER/GMS Output File	
File Name	**Description**
Printed Output	Text file containing a summary of the output.
Pressure Head	Text or binary file containing the computer pressure heads. Used for postprocessing or as initial conditions for a subsequent analysis.
Moisture Content	Text or binary file containing the computed moisture content at nodes. Used for postprocessing.
Velocity	Text or binary file containing the computed Darcian velocities. Used for postprocessing.
Concentration	Text or binary file containing the computed concentrations. Used for post-processing or as initial conditions for a subsequent analysis.

Brooks-Corey formulations

The Brooks-Corey formulations for moisture content, relative hydraulic conductivity, and water content are defined below.

The moisture content θ, dimensionless, is defined as :

$$\theta = \theta_r + (\phi - \theta_r) * \left(\frac{h_b}{h}\right)^{\lambda} \qquad (2)$$

and the relative hydraulic conductivity K_r (dimensionless) is defined as:

$$K_r = \left(\frac{h_b}{h}\right)^{(2+3\lambda)} \tag{3}$$

and the water content ($C_m(h)$, L^{-1}) is defined as:

$$\begin{aligned} C_m(h) &= -(\phi - \theta_r) * \left(\frac{\lambda}{h_b}\right) * \left(\frac{h}{h_b}\right)^{-(\lambda+1)} \quad for \quad h \le h_b \\ C_m(h) &= 0 \quad for \quad h > h_b \end{aligned} \tag{4}$$

where

θ_r = residual moisture content (dimensionless)

ϕ = porosity (dimensionless)

h_b = bubbling or air-entry pressure (L)[1]

h = pressure head (L)

λ = pore-size-distribution index, a function of soil texture (dimensionless)

To evaluate the parameters used in Equations 2-4, published values for soil types, which match site soil characteristics are normally used. Saturated hydraulic conductivity data (Site Subsurface Soil, Table 1 of this chapter) were ranked into six classes of materials to match the Brooks and Corey (1964) parameters given in Table 4 that follows. These parameters were used in the above equations to calculate required unsaturated soil input data. An alternative approach is to determine these parameters from the best-fit line through the data points of pressure head versus effective saturation. The slope of the line represents λ, and its intercept at full saturation represents h_b. However, collection of appropriate data is frequently impractical because of cost and time constraints. The use of Brooks and Corey parameters provides a reasonable input for the model in the absence of adequate site data.

Transport equations

The governing equation for the transport part of FEMWATER is based on continuity of mass and advection/diffusion laws:

[1] The upper case letters, L, T, and M are used to denote generic length, time, and mass units.

Table 4
Brooks and Corey (1964) Parameters Used in the Model

Soil Type	Saturated Hydraulic Conductivity K, m/day	Site Hydraulic Conductivity Range, m/day	ϕ	θ_r	$-h_b$	λ
Unconsolidated sand	8.5	9.20 - 11.4	0.424	0.09	0.114	4.4
Sand	8.2	6.2	0.435	0	0.196	0.84
Fine sand	2.1	2.3 - 3.2	0.377	0.06	0.82	3.7
Columbia sandy loam	0.7	1.18 - 1.6	0.496	0.11	0.85	1.6
Touchet silt loam	0.22	0.02 - 0.11	0.43	0.1	1.45	1.7
Hygiene sand stone	0.15	0.0031	0.25	0.13	1.06	2.9

$$\theta_w \frac{\partial C}{\partial t} + \rho_b \frac{\partial S}{\partial t} + V \cdot \nabla C = \nabla \cdot (\theta_w D \cdot \nabla C) - \kappa(\theta_w C + \rho_b S) + QC_{in} - \left[\frac{\rho^*}{\rho} Q - \frac{\rho_0}{\rho} V \cdot \nabla(\frac{\rho}{\rho_0})\right] C \quad (5)$$

$$S = K_d C \quad \text{linear isotherm}$$

$$S = \frac{S_{max} K_L C}{1 + K_L} \quad \text{Langmuir isotherm} \quad (6)$$

$$S = K_F C^n \quad \text{Freundlich isotherm}$$

where

θ_w = moisture content (dimensionless)

C = aqueous phase concentration (M/L^3)

t = time

ρ_b = bulk density of medium (M/L^3)

S = solid (or adsorbed) phase concentration (M/M)

V = flow velocity (L/T)

∇ = del operator

D = dispersion coefficient tensor (L^2/T)

κ = decay rate (1/T)

Q = volume flow rate per unit volume of source or sink (1/T)

C_{in} = source or sink concentration

ρ^* = density of injected fluid (M/L^3)

K_d = distribution coefficient (L^3/M)

S_{max} and K_L = maximum absorbed concentration allowed in medium (M/M) and the constant coefficient in the Langmuir nonlinear isotherm, respectively

K_F and n = coefficient and power constant for Freundlich nonlinear isotherm, respectively

The dispersion coefficient tensor D (L^2/T) in Equation 4 is given as:

$$\theta_w D = \alpha_T |V| \delta + (\alpha_L - \alpha_T) \frac{VV}{|V|} + \alpha_m \theta_w \tau \delta \quad (7)$$

where

$|V|$ = magnitude of vector velocity V (L/T)

δ = Kronecker delta tensor

α_T = lateral dispersivity (L)

α_L = longitudinal dispersivity (L)

α_m = molecular diffusion coefficient (L^2/T)

τ = tortuosity

Model limitations

Major assumptions and limitations of FEMWATER include the following: (a) single constituent transport, thus intersolute reactions cannot be simulated, (b) abiotic and microbial degradation is treated with a first-order decay model, (c) adsorption coefficient and decay rates can be assigned for different subsurface materials; however, rate constants do not change during simulation time, (d) contaminant sorption is instantaneous and reversible, and the adsorbed phase is in local equilibrium, and (e) microbial biomass production, fate and transport of electron

acceptors, and cometabolic degradation are not included in FEMWATER. A new code (3DFATMIC) has been developed based on FEMWATER, which includes options for more detailed calculations; however, the input data requirements are more complex (U.S. Environmental Protection Agency 1997a).

For the LAAP site, the above assumptions were substantiated by introducing some simplifications of the site characteristics. For more details, the reader is referred to FEMWATER model theory documentation (Lin et al. 1997).

Model Construction

FEMWATER requires basic hydrogeologic and chemical data for its simulations. The input data include hydraulic conductivity, porosity, hydraulic gradient, infiltration rate, initial and boundary conditions, distribution (partition) coefficient, and decay rates. The distribution coefficient K_d relates the sorbate and solute for linear isotherms.

Modeling domain

In plane view, the modeling domain is bounded by D-Line on the west and Pearl Harbor Avenue on the east. In the vertical direction, the modeling domain includes the Upper Terrace and the Lower Terrace aquifers (Figures 3 and 4). The three-dimensional mesh for this site (Figure 2) was constructed by first dividing the surface domain into uniform triangular elements and then adding the subsurface layers into the mesh. For detailed information on mesh generation, the reader is referred to the GMS users's manual (1996).

The modeling domain does not cover any physical boundaries such as streams. However, FEMWATER requires numerical flow data at the boundaries. The water-level elevation data collected at the monitoring wells were used to generate boundary data for the model. The water-level data were collected in both the Upper Terrace and the Lower Terrace aquifer. Two-dimensional interpolation tools of GMS were used to create contours of water-level elevation in both aquifers. The intersection between these contours and boundaries provided the boundary condition values.

Other required parameters in FEMWATER include convergence criteria and coefficients of numerical solution techniques. One of the parameters that controls the amount of leachate entering the unsaturated zone is the infiltration rate. The infiltration rate is usually calculated from precipitation data and soil characteristics. The precipitation data collected at Minden was used to estimate infiltration rates (Figure 5).

Another parameter, hydrodynamic dispersion (i.e., the spreading and mixing caused by mechanical dispersion), was introduced in FEMWATER in terms of dispersivity (α). The field values for dispersivity normally are unknown and difficult to obtain. These parameters are strongly scale dependent and vary with site dimensions (Electric Power Research Institute 1985). In this study, dispersivities

were adopted from values reported for other sites with similar characteristics. Reported values for dispersivities include 21.3 m longitudinally and 4.27 m transversely for a glacial outwash aquifer consisting of beds of fine and coarse sand, gravel, and silt in Long Island, NY (Pinder 1973), and 0.6 m longitudinally for the Bunter Sandstone aquifer near Mansfield, England (Oakes and Edworthy 1976).

In this application, the following values for dispersivity were used because the measured values were unavailable. Adjustment of the dispersivity values during model calibration is possible.

$$\begin{aligned} \alpha_L &= 21.3\,\text{m} \approx 70\,\text{ft} \\ \alpha_T &= 4.27\,\text{m} \approx 14\,\text{ft} \end{aligned} \tag{8}$$

Retardation factor

In mathematical modeling, contaminant adsorption is based on the concept of the retardation factor R as:

$$R = \frac{u}{u_s} \tag{9}$$

where

u = mean water velocity

u_s = mean chemical (solute) velocity (L/T)

The retardation factor provides a general indication of the mobility of a contaminant in the soil. Hartley and Graham-Bryce (1980) have shown that R (dimensionless) is equivalent to the ratio of total concentration (C_T, M/L^3) to dissolved concentration (C_w, M/L^3) of contaminant.

$$\begin{aligned} C_t &= \text{Dissolved} + \text{Adsorbed} \\ C_t &= C_w\, \phi\, S_w + C_s\, \rho_B \end{aligned} \tag{10}$$

where

C_s = concentration of chemical adsorbed to the solid particles (M/M)

ρ_B = bulk density (M/L^3)

ϕ = porosity

S_w = water saturation (volume of water/volume of voids)

ϕS_w = moisture content (volume of water/bulk volume)

If linear equilibrium adsorption is assumed as described earlier, then the retardation factor R because of adsorption is given as:

$$R = \frac{C_w \phi S_w + C_s \rho_B}{C_w \phi S_w} = 1 + \frac{C_s \rho_B}{C_w \phi S_w} = 1 + \frac{K_d \rho_B}{\phi S_w} \quad (11)$$

Hence, the retardation factor is a function of chemical sorption (K_d) and soil properties (ρ_B, ϕ, S_w).

Adsorption coefficients

Results from laboratory batch testing for adsorption of explosives (TNT and RDX) to LAAP soils were used to develop input data for modeling purposes. The initial exposure of uncontaminated soil to TNT and RDX could represent the soil response to the contamination front as it migrates through the aquifer. The rates of laboratory-measured sorption of TNT and RDX were about two orders of magnitude faster than the microbial mineralization. Pseudo-equilibrium of TNT and RDX with LAAP soils was reached within a few days. After equilibrium, the removal rate was dominated by the microbial activity. Adsorption coefficients for TNT and RDX in LAAP soils are presented in Table 5. The K_d values for TNT ranged from 0.08 to 0.33 L kg^{-1} depending on the type of soil. For RDX, the values ranged from 0.21 to 0.33 L kg^{-1}. An average value of 0.228 L kg^{-1} for TNT and 0.30 L kg^{-1} for RDX were used for the modeling.

Table 5
Explosives Adsorption Coefficients (K_d, L/kg) for LAAP Aquifer Soils and Regression Coefficient (r^2)[1]

	ML Soil		SP-SM Soil		CL Soil		SM Soil	
Compound	K_d	r^2	K_d	r^2	K_d	r^2	K_d	r^2
TNT	0.33	0.96	0.23	0.99	0.27	0.92	0.08	0.90
RDX	0.21	0.95	0.33	0.97	0.33	0.83	0.33	0.95

[1] Batch tests conducted under aerobic conditions.

Decay rates

A critical input to the model is an estimate of the rate of contaminant decay or removal that is reflective of the dominant biogeochemical pathways at the site. Direct measurement of in situ rates of microbial degradation currently is not possible. One approach to measure the degradation rates is to sample aquifer sediments and monitor contaminant disappearance as a function of time using batch or column reactors operated under controlled laboratory conditions. Alternatively, the use of radiorespirometric techniques can be applied to measure microbial degradation potential in the laboratory. Radiorespirometry indicates the potential for complete mineralization. The actual rate in the groundwater would differ from laboratory

tests because of inherent differences in the biomass, temperature, electron acceptors, and mass transfer limitations. The laboratory approach introduces uncertainties (Madsen 1991) and may greatly overestimate rates of microbial metabolism in some groundwater system (Chapelle and Loveley 1990). In the batch tests, mass transport limitations are eliminated, and the mixing conditions promote more effective contact between aquifer materials and groundwater than can be achieved in the field. Laboratory tests represent the potential rate of degradation in the absence of mass transport limitations.

The decay rates from the laboratory batch studies and radiorespirometric studies on the LAAP soils are given in Tables 6 and 7. The batch tests were conducted using uncontaminated LAAP soils exposed to LAAP groundwater contaminated with TNT and RDX (90 g soil/360 g water). The apparent rate constants ranged from 0.01 to 0.03 $days^{-1}$, with corresponding half-lives on the order of month (Table 6). The heterogeneity of the soil and the complexity of physical and chemical interactions in a multicomponent system render the generation of these values difficult. The radiorespirometric data are based on mineralization of a 30-percent slurry of LAAP soil exposed to either TNT (2 mg L^{-1}) or RDX (21 mg L^{-1}). The apparent rate constants from radiorespirometry are one to two orders of magnitude lower than the batch-testing results with half-lives ranging from 1 to 10 years. In general, the highest rates of removal were associated with clay soils.

Table 6
Summary of Apparent First Order Removal Rate Constants for Uptake of TNT and RDX from Groundwater on Uncontaminated LAAP Soils[1]

	TNT		RDX	
Soil Type	Decay Rate Constant, k, day^{-1}	Half-Life, days	Decay Rate Constant, k, day^{-1}	Half-Life, days
Sandy silt ML	0.014	48	<0.002	>350
Sandy silt (SP-SM)	0.014	48	<0.002	>350
Lean clay (CL)	0.034	20	<0.002	>350
Silty sand (SM)	0.017	42	<0.002	>350

[1] Water source was MW085U; results are from batch tests conducted under anaerobic conditions; initial concentrations were approximately 8 and 10 mg TNT and RDX L^{-1}, respectively.

The capacity of the soil to support biological degradation varies slightly over the site because of physical chemical properties of the soils. Rate constants determined in the laboratory on soils from the site have several inherent limitations:

Table 7
Summary of Apparent First Order Microbial Mineralization Rate Constants for Degradation of TNT and RDX in LAAP Soils [1]

Soil Type	TNT		RDX	
	Radiorespirometry Rate Constant, k, day^{-1}	Half-Life, days	Radiorespirometry Rate Constant, k, day^{-1}	Half-Life, days
Sandy silt ML	5.7×10^{-4} to 2.2×10^{-3}	320 to 1,220	1.8×10^{-4}	3,850
Sandy silt (SP-SM)	4.6×10^{-4}	1,510	5×10^{-4}	1,390
Lean clay (CL)	$<1 \times 10^{-4}$	>3,900	$<1 \times 10^{-4}$	>3,900
Silty sand (SM)	$<1 \times 10^{-4}$	>3,900	2×10^{-3}	323

[1] Radiorespirometry tests were run at 23.3 ± 3.2 °C under aerobic conditions. Initial aqueous phase concentrations were 2 and 21 mg TNT and RDX L^{-1}, respectively.

(a) Detection limits of the tests may be above actual in situ decay rates based on modeling of the historical contaminant data. The detection limit for measurement of microbial rate constants using radiorespirometry was limited to 10^{-4} day $^{-1}$, while for batch tests the rate constant detection limit was about 10^{-3} day $^{-1}$. The model suggested a rate of 10^{-5} day^{-1}. (b) Rates from radiorespirometry are based upon optimal microbial conditions that may exist only sporadically within the aquifer; therefore, this rate is likely to exceed what is typical of the site as a whole. (c) Batch tests also simulate optimal partitioning and leaching conditions that are more likely to typify quasi-equilibrium conditions in the site. These rates are also likely to exceed actual rates within the site.

In spite of the limitations of these data, laboratory tests provide the best approximation of site conditions and should be taken into account by the model. The batch tests would simulate uptake rates at the edge of the plume where uncontaminated soils are initially exposed to TNT and RDX (Table 6). Because the LAAP site has a history of over 40 years of exposure to explosives, microbial degradation is likely to be the dominant factor controlling the removal rate. Therefore, the decay rates from radiorespirometry were closer to the decay values used in the model.

The field concentration data represent the change in contaminant concentration at specific locations at the site and incorporate all removal mechanisms and mass-transport limitations. The decay rates used in the model were based on the radiorespirometry results (Table 7) and model calibration using field concentration data. The values used in the model were 10^{-5} day^{-1} (half-life of 190 years) for TNT and 8.13×10^{-6} day^{-1} (half-life of 233 years) for RDX.

Initial Flow and Concentration Distributions

The modeling domain consisted of Area P and vicinity. The modeling focused on transport of TNT and RDX. Initial conditions of flow and contaminant concentration play a major role in model outcomes. Different numerical techniques available in GMS were compared to establish realistic initial flow and mass-concentration distributions at the site. The amount of explosives originally dumped at the site is unknown. Therefore, for the modeling exercise described here, the first round of the water-level elevations and concentrations (TNT and RDX) data collected in February 1996 were used as initial conditions for flow and mass transport calculations of the model, respectively. The GMS was used to interpolate/extrapolate the data for all points of the numerical mesh system.

Calibration

Numerical flow models are calibrated by adjusting values of hydraulic conductivity, boundary conditions, and recharge rates and their distribution so that a reasonable match between the simulated and measured hydraulic head is achieved in spite of any possible measurement errors. The transport model is calibrated by adjusting adsorption rates, decay rates, and dispersion parameters.

For the LAAP site, the calibration process was carried out by manual trial and error. The calibration included varying parameters such as flow boundary conditions, hydraulic conductivity distributions, and infiltration rates until a reasonable match between observed and simulated flow conditions at monitoring wells were obtained. GMS 2.0, used here, has an option called Gages Tool that was used to compare the simulated and measured results (in GMS 2.1, Map Module has a similar function). Figure 6 illustrates the location of the gauges (monitoring wells) used in the model calibration. The simulated and measured water-level elevation and TNT and RDX concentration at selected monitoring wells were compared. The simulated and measured total head (water-level elevation) at MW037U (downstream and screened in the Upper Terrace aquifer), MW138L (downstream and screened in the Upper Terrace aquifer), and MW142U (upstream and screened in the Lower Terrace aquifer) are shown in Figures 7 through 9. As illustrated in these figures, the model is able to simulate the head at these locations even though the site hydrogeology is fairly complex. The maximum difference between the simulated and measured head is about 1 m.

The hydraulic conductivity of the site has a major effect on the flow calibration compared with the other parameters mentioned earlier. Therefore, additional data to define the spatial variability of hydraulic conductivity at the LAAP site would help in reconciling the simulated results with the measured values.

The accuracy of the transport model of explosives is controlled by both flow and chemical data. After the flow model is calibrated, the transport model must be calibrated using site-specific chemical input data. The model was calibrated using this rate. A representative simulated and measured concentration of TNT and RDX

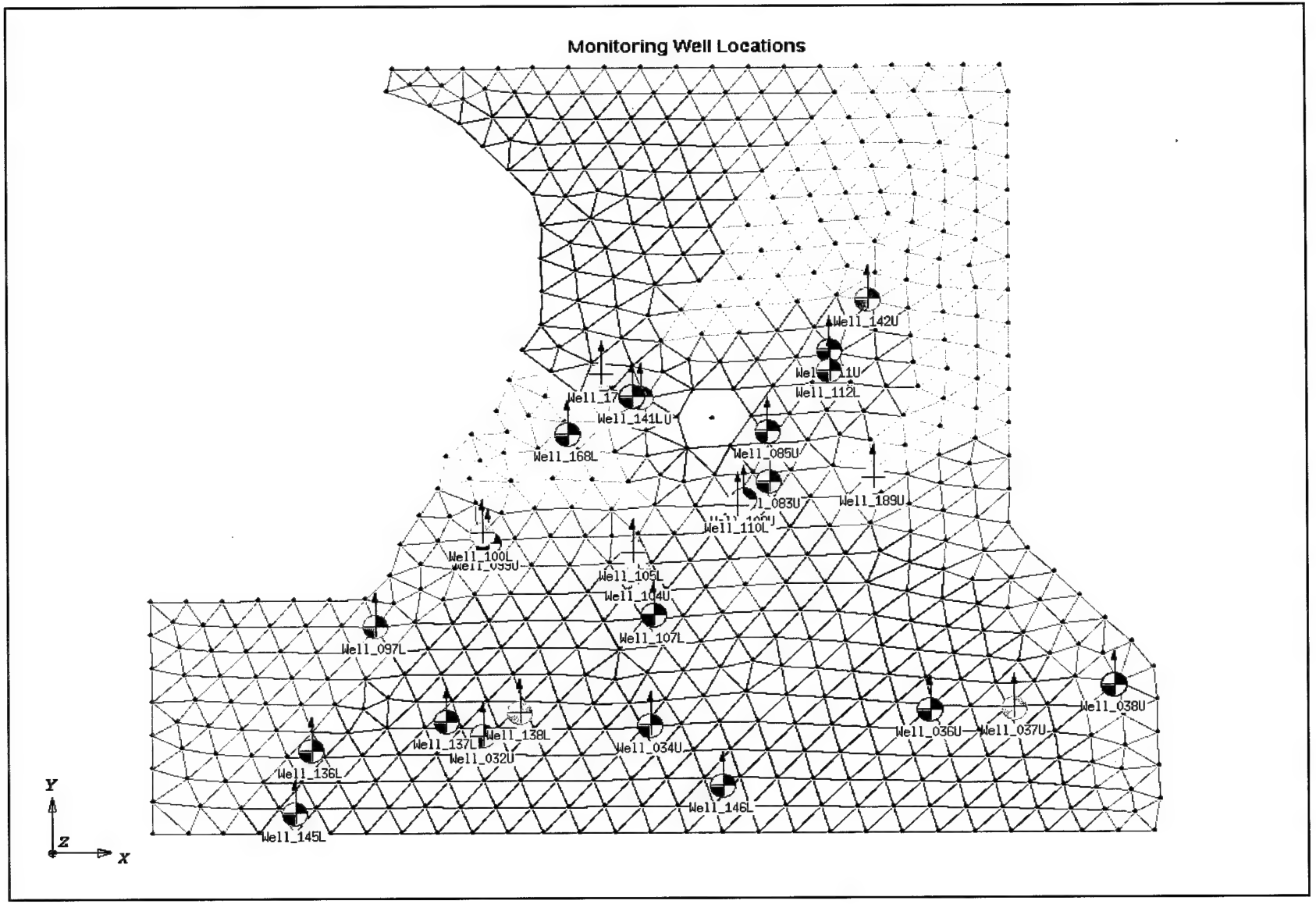

Figure 6. Locations of monitoring wells (gauges in GMS 2.0) screened in different aquifer layers

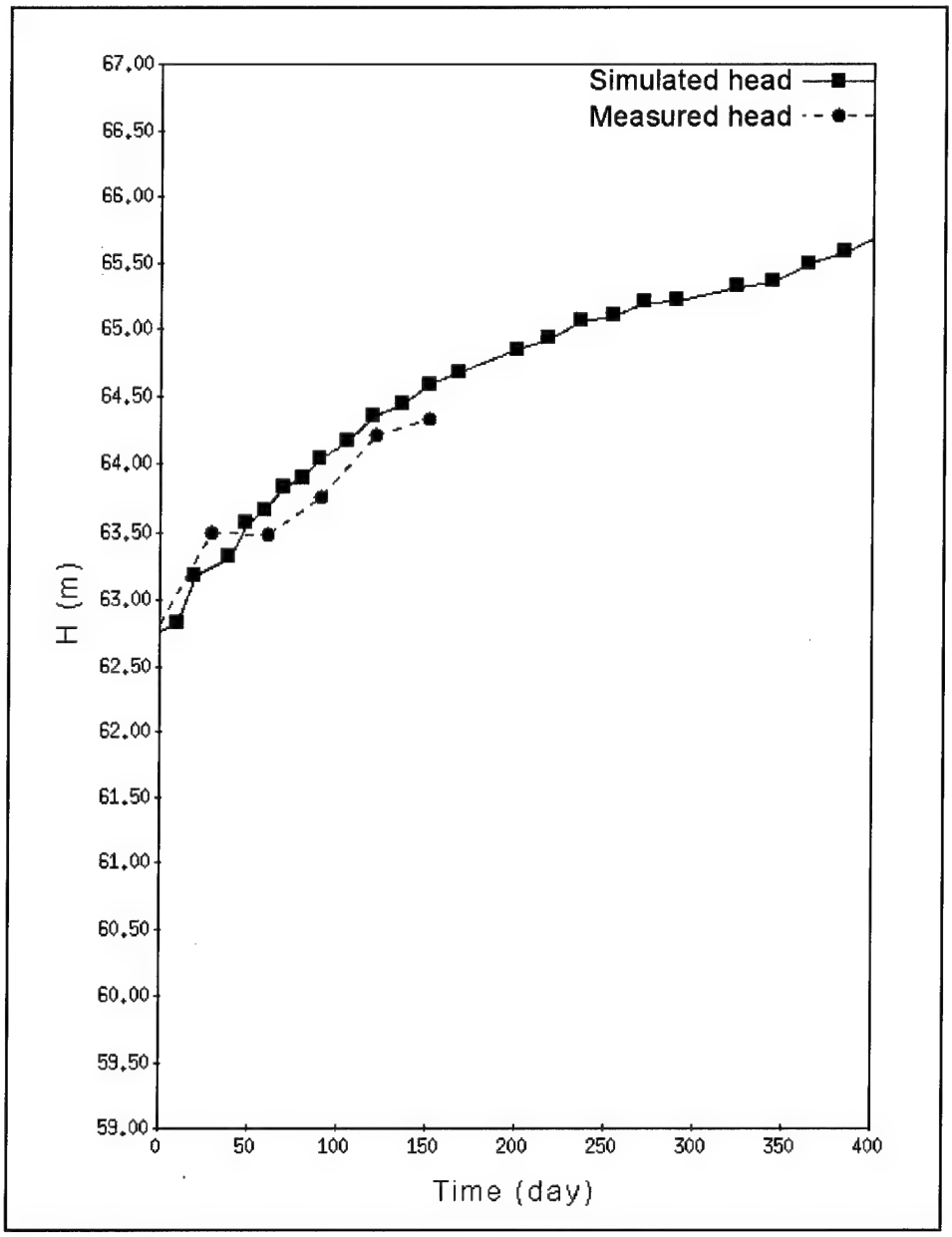

Figure 7. Comparison between measured (--•--) and simulated (--■--) head (ft, above MSL) in MW037U screened in the upper aquifer

over 400 days of simulations are given in Figures 10 (representing a high concentration of TNT) and 11 (representing a high concentration of RDX). As illustrated in Figure 10, the difference between the simulated and measured TNT concentrations are reasonable and displays similar trends of reduction. For RDX (Figure 11), at the beginning of the simulation, the differences between the simulated and measured concentrations are about 2,000 $\mu g\ L^{-1}$, but as the time advances, the differences were reduced. One reason for this discrepancy is that interpolation/ extrapolation of the initial conditions might not have captured the actual measured

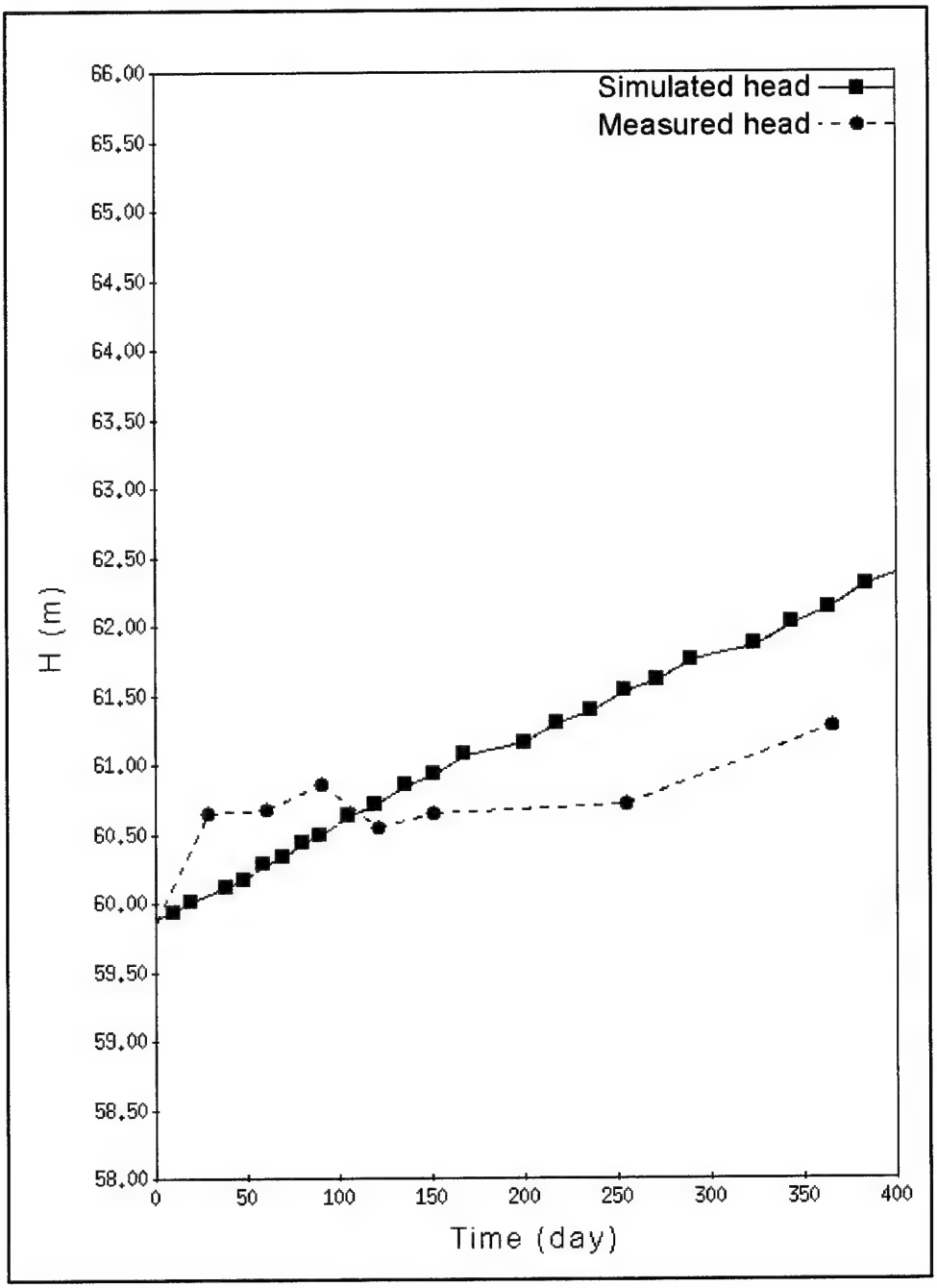

Figure 8. Comparison between measured (--•--) and simulated (--■--) head (above MSL) in MW138L screened in the lower aquifer

initial concentration of RDX at this monitoring well. The simulated and measured RDX concentrations have similar trends of reduction, which affects the long-term prediction. The long-term prediction of the plume is important in evaluating the ultimate outcome of natural attenuation at this site. The calibrated models were applied to development of long-term predictions of TNT and RDX fate and transport.

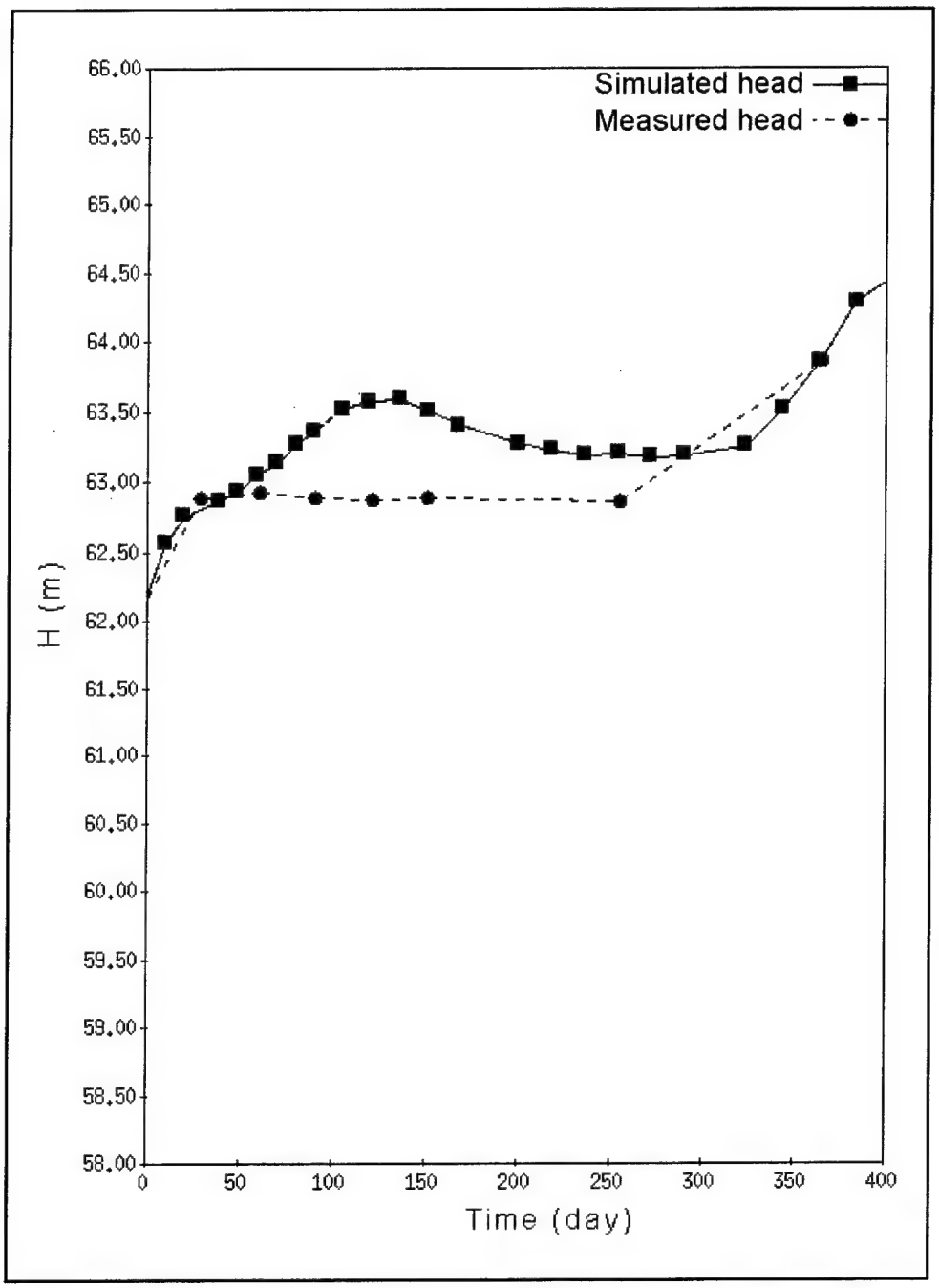

Figure 9. Comparison between measured (--•--) and simulated (--■--) head (above MSL) in MW142U screened in the upper aquifer

Sensitivity Analysis

The sensitivity of the model simulations and predictions coupled with the desired level of accuracy determines the level of detail required for field and laboratory measurements that are used for model input. A model that is not

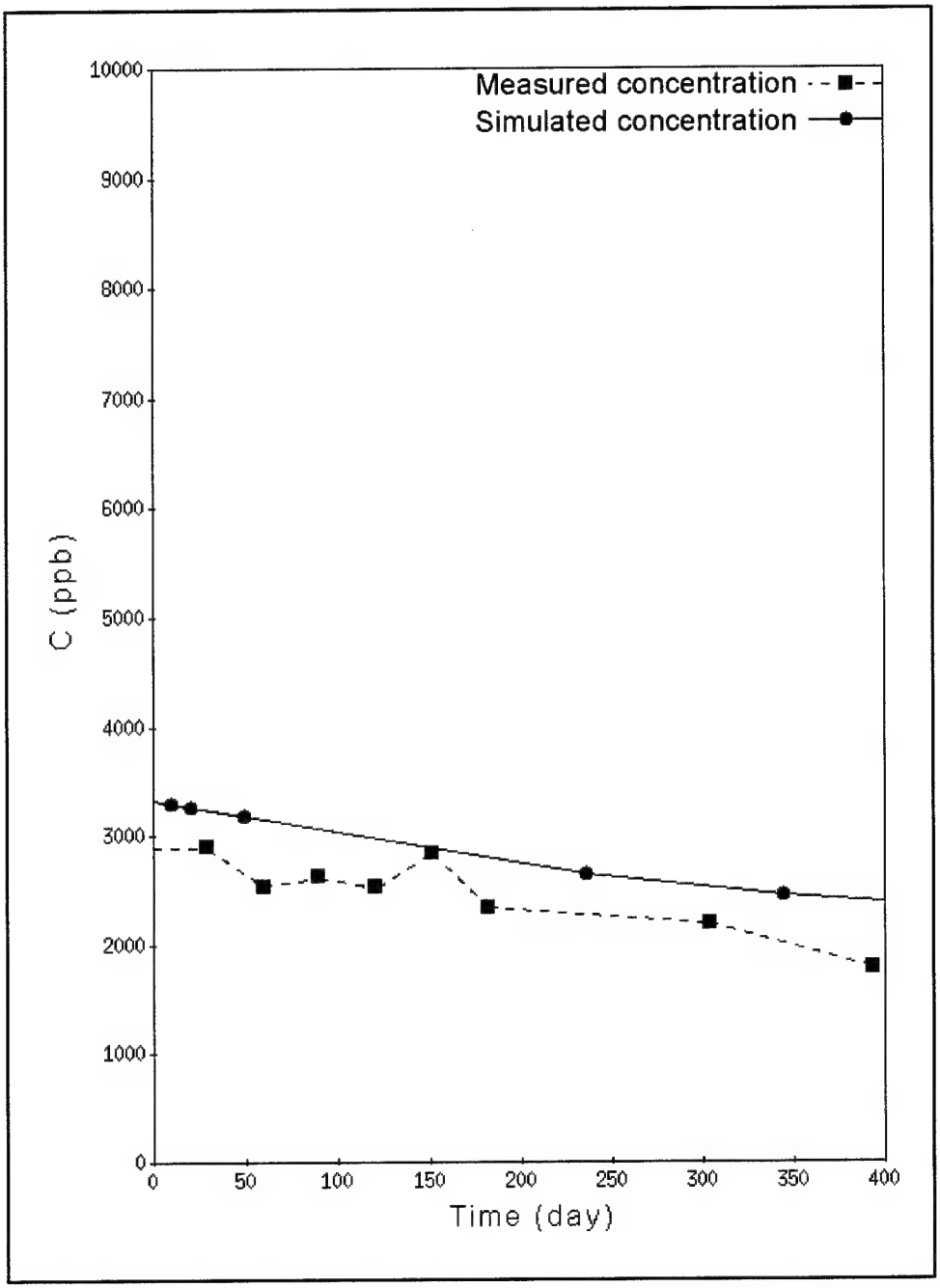

Figure 10. Simulated (--●--) and measured (--■--) TNT concentration in MW083U versus time

sensitive to a specific input parameter may produce the same output regardless of input changes. On the contrary, if a model is sensitive to a parameter, the model results may alter significantly by a small change of that parameter. Therefore, more efforts are needed to estimate the value of the sensitive parameter correctly.

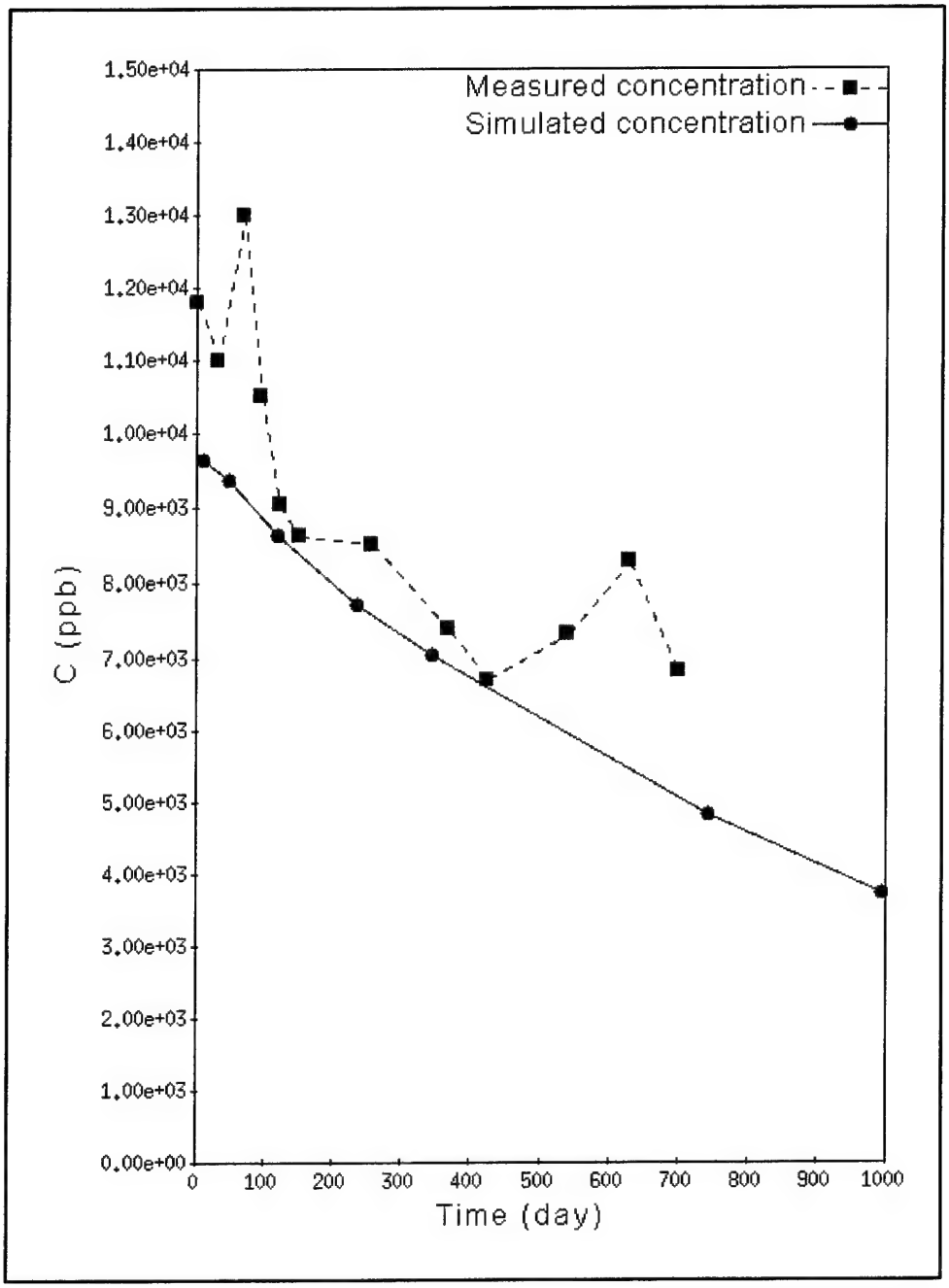

Figure 11. Simulated (--●--) and measured (--■--) RDX concentration in MW85U versus time

Sensitivity analysis for a particular model may be performed in several ways. The simplest approach, which is applicable to all numerical models and exercised here, is to determine the effect on the output by systematically changing the value of each input parameter.

The model was evaluated for its sensitivity to changes in the values of hydraulic conductivity, adsorption coefficient, and decay rate. The hydraulic conductivities were increased and decreased by an arbitrary value of 10 percent, and the results for TNT concentration were compared. Figure 12 shows the time series of measured TNT concentrations along with simulated results of three scenarios with different hydraulic conductivities at MW083U screened in the Upper Terrace aquifer. Increasing or decreasing the hydraulic conductivity by 10 percent did not significantly impact the modeled results. The reason for the apparent lack of sensitivity to the hydraulic conductivity is the relatively low conductivity associated with this site. This indicates that the model was not sensitive to changes in hydraulic conductivity at the LAAP site.

The sensitivity of the model to the adsorption coefficient is shown in Figure 13. One order of magnitude change of the adsorption coefficient from 0.23 to 2.3 L kg^{-1} caused about 40 percent change in TNT concentration at the end of 1,000 days of the simulations. Increased adsorption results in lower overall decay of TNT concentration in the groundwater during 1,000 days of the simulation for the range of values considered here. The extent of adsorption on LAAP soils is quite low relative to other soil types. This indicates that the model is sensitive to the adsorption coefficient.

The sensitivity analysis of the model to decay rate was tested by varying the decay rate from 10^{-5} to 10^{-4} day^{-1}, while holding adsorption and conductivity constant. About 12 percent change was observed in the predicted TNT concentration at the end of 1,000 days simulations (Figure 14). This indicates that the model is moderately sensitive to the decay rate. Refinements in the understanding of the interplay between adsorption and degradation would improve the accuracy of the model simulations.

Predictive Simulations

Prediction of the fate and transport of TNT and RDX at the LAAP site requires calculating future flow and transport patterns derived from available historical data. The future boundary and other required model conditions are a mathematical statement of specific hypotheses, based on past experiences.

To predict changes that might occur in the distribution of TNT and RDX at the site for a 20-year time interval, several assumptions were imposed on the model: (a) no additional source of contamination is added into the site, (b) infiltration rate stays constant throughout the time period under investigation, (c) flow-boundary conditions recur every year, and (d) no recharge or discharge through pumping occurs during the simulations. Adsorption coefficients of 0.23 and 0.3 L kg^{-1} and degradation rates of 10^{-5} day^{-1} and 8.13×10^{-6} day^{-1} were used to develop a 20-year prediction of the fate and transport of TNT and RDX at LAAP, respectively.

When initial conditions (Figures 15 and 17 for TNT and RDX, respectively) are compared with simulations for 20 years (Figures 16 and 18) (for TNT and RDX,

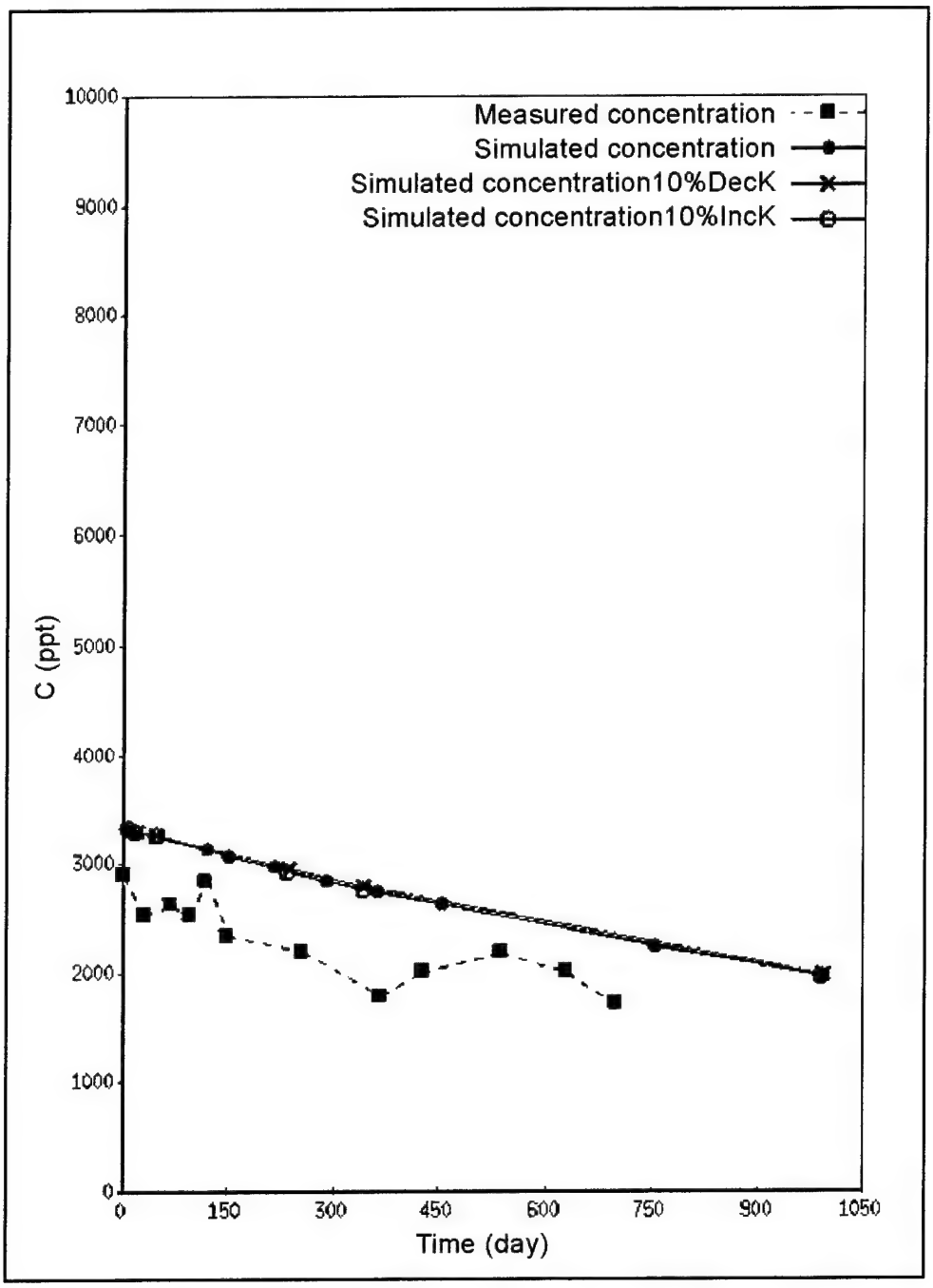

Figure 12. Time series of TNT concentration for measured calibrated model, with 10-percent increase of conductivity, and with 10-percent decrease of conductivity, respectively (Original data were from MW083U)

respectively), the areal extent of both plumes is diminishing. The highest concentration is reduced from 10,500 to 250 $\mu g\ L^{-1}$ for TNT and 23,200 to 620 $\mu g\ L^{-1}$ for RDX. The predicated results should be updated, adjusted, and verified as new data become available. For example of the iterative way in which a model prediction can be improved as new information is obtained, the reader is referred to Jorgensen (1981).

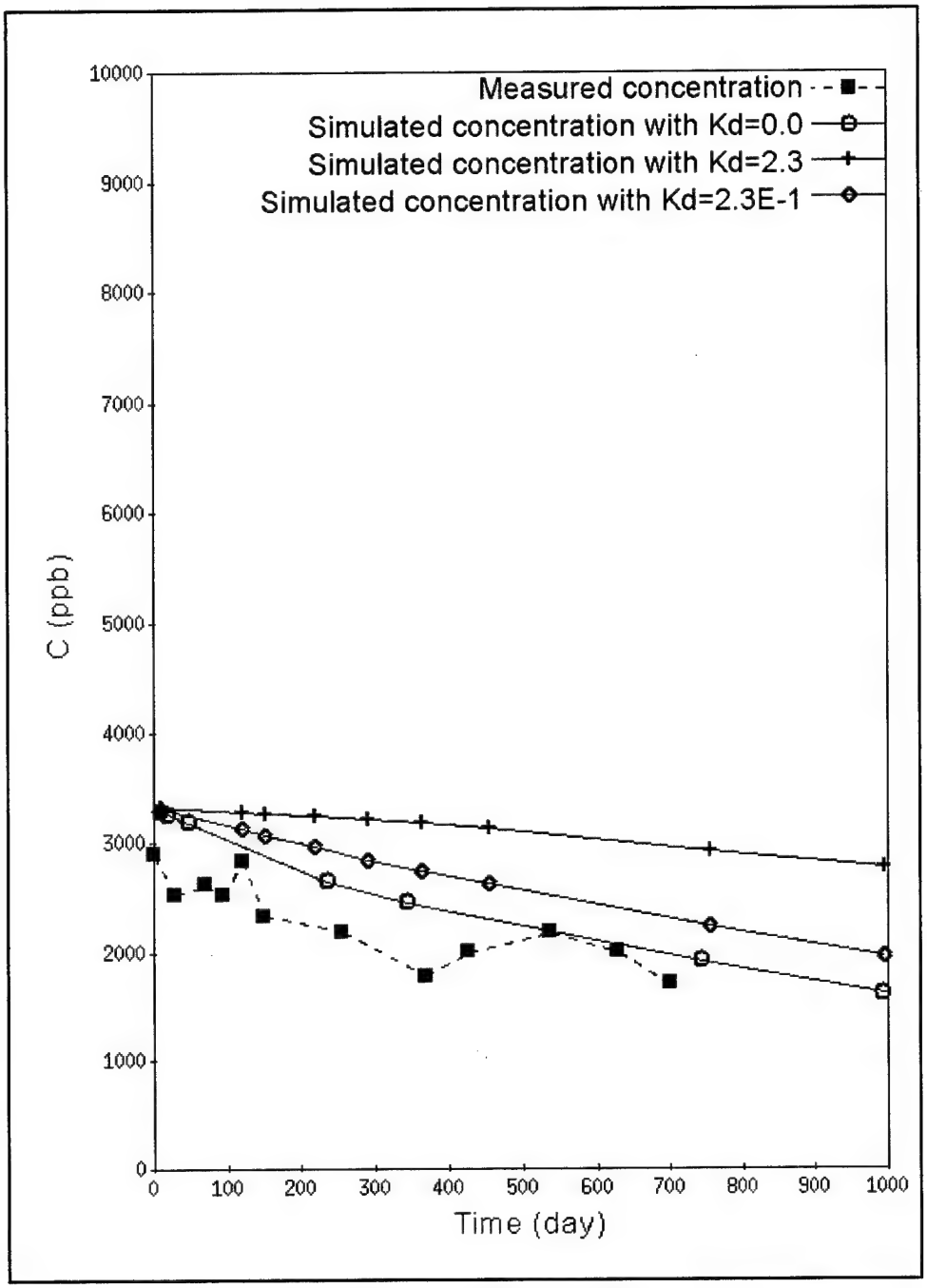

Figure 13. Time Series of TNT concentration for measured and calibrated model with different adsorption coefficients (L kg^{-1}) as shown above (Original data were from MW083U)

Contaminant Mass

One of the three lines of evidence for natural attenuation (U.S. Environmental Protection Agency (EPA) 1977b) is to demonstrate reduction of contaminant mass over time. Contaminant masses were calculated using the measured and predicted

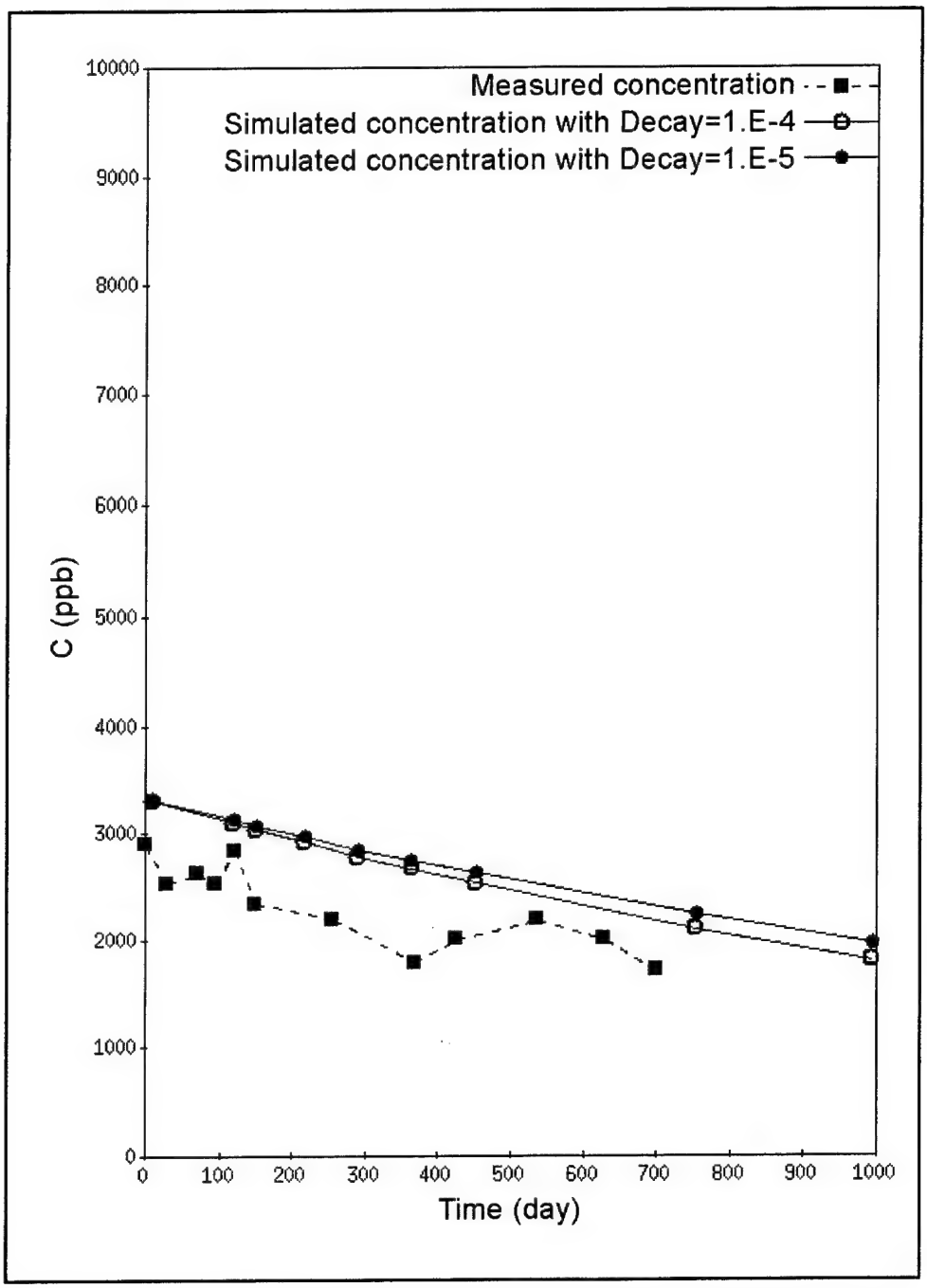

Figure 14. Time series of TNT concentration for measured and calibrated model with different first-order decay rates (1.E-4 and 1.E-5 1/day) and fixed adsorption coefficient of 2.3E-1 L kg^{-1} as shown above (Original data were from MW083U)

TNT and RDX concentrations along with iso-surface volume calculation of GMS (1996). The estimated initial (February 1996) masses of TNT and RDX are 52 and 78 metric tons, respectively. The predicted values after 20 years are 1 and 0.8 metric tons for TNT and RDX, respectively. The accuracy of these numbers is

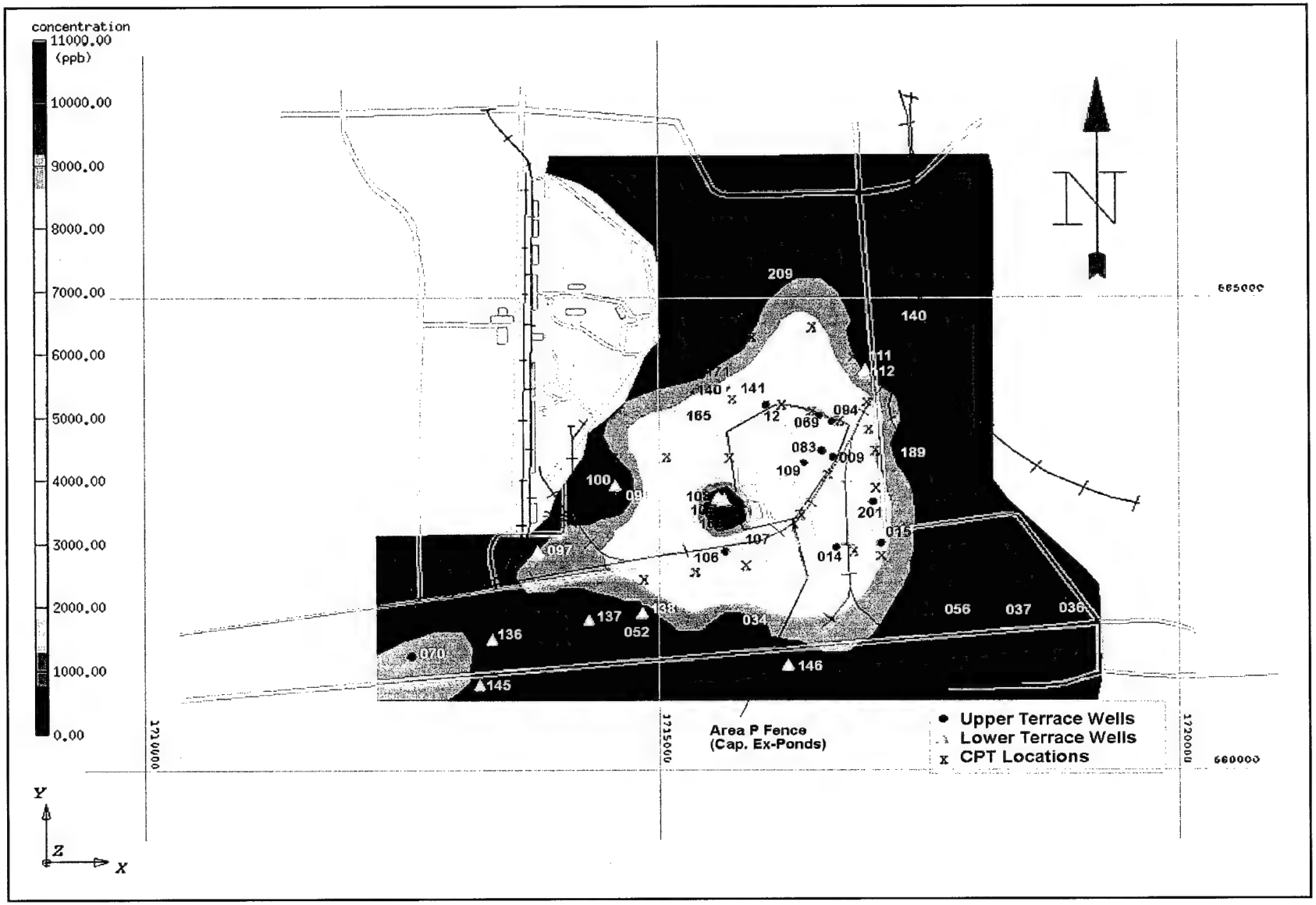

Figure 15. Initial (February 1996) distribution of TNT concentration

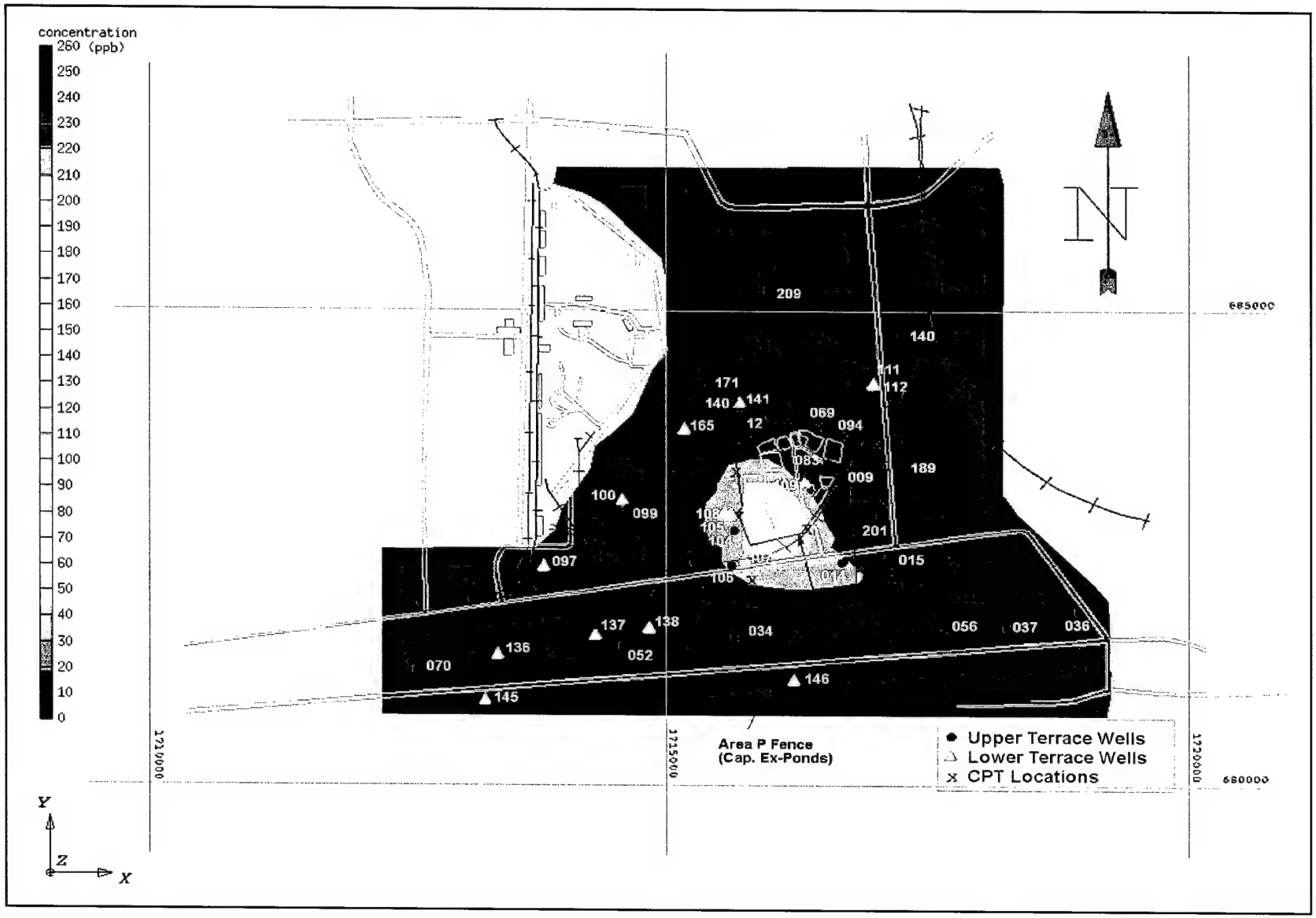

Figure 16. Predicted distribution of TNT plume after 20 years

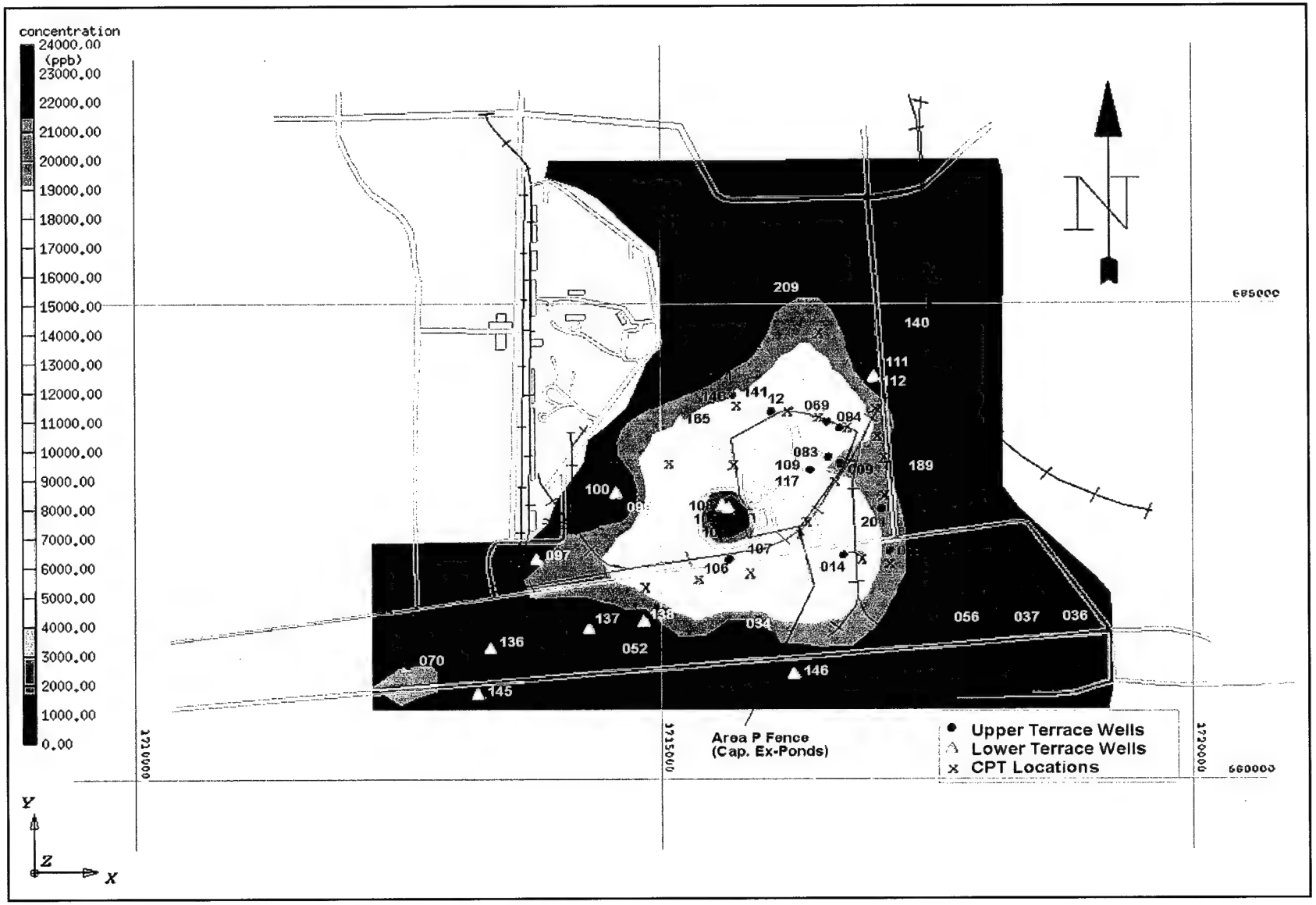

Figure 17. Initial distribution of RDX concentration (February 1996)

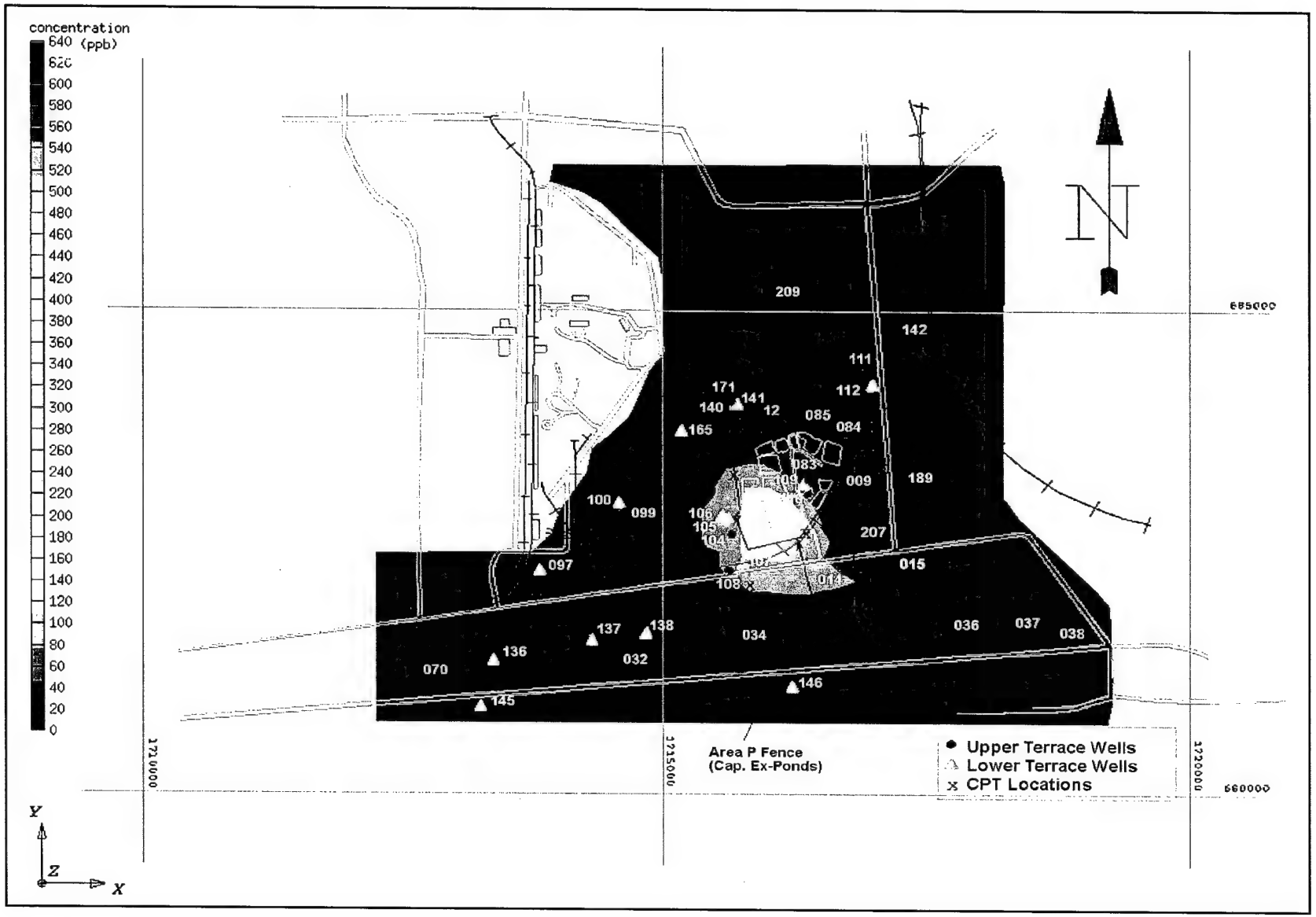

Figure 18. Predicted distribution of RDX concentration after 20 years

controlled by the assumptions and limitation of FEMWATER and iso-surface calculations of GMS.

Conclusions

GMS provided efficient numerical tools to integrate and analyze complex and multidisciplinary field data into simpler graphic forms that were used to illustrate fate and transport of contaminant plumes. The measured and simulated flow data indicated slow subsurface flow at the LAAP site, because of the low-permeability media and low-hydraulic gradients. The TNT and RDX plumes are virtually static. The simulated flow directions are consistent with the direction of explosives plume propagation. Although, some of the contaminant may move from the Upper Terrace aquifer to the Lower Terrace aquifer, the total mass of contaminants are anticipated to decline.

The simulated results indicated that explosives at LAAP may be reduced naturally without posing any threat to offsite receptors. The supporting factors for natural attenuation of explosives at the site including low degree of sorption, low values of the hydraulic conductivity, and low rate of mineralization were evaluated and illustrated. Even though the reduction process is very slow, the plume is confined to a limited area and is not moving significantly. The results of contaminant mass calculations indicated that the initial mass of TNT and RDX will be reduced significantly during 20 years. The sensitivity analysis suggested that the important model input parameters are the adsorption coefficient rates and the biodegradation rates. The predicted results should be adjusted and the calibration processes repeated as new data become available.

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6 Conclusions

Declining concentrations of explosives over the 2-year monitoring period were documented at LAAP. Results support the first line of evidence required under EPA guidance for verification of monitored natural attenuation, i.e., declining contaminant mass. Methods were developed to optimize accuracy and minimize varibility between sampling events, so that trends in concentration over time were readily demonstrated and reliable. None of the geochemical characteristics of the site correlated with explosives concentrations. Therefore, monitoring geochemical parameters provided no evidence of natural attenuation processes at LAAP. Sampling methods developed at LAAP were verified by application at JAAP. Although the sampling period at JAAP was limited to 9 months, about 20 percent of the wells exhibiting concentrations of explosives above detection limits showed significant declines. Geochemical parameters were unrelated to explosives concentrations as observed at LAAP.

Definition of the contaminant plume was refined by cone penetrometry sampling at both sites. By coupling rapid laboratory "turn-around" with placement of the CPT, efficiency was optimized while minimizing analysis of uncontaminated samples beyond the plume. Lithology and contaminant data were used in the site conceptual and numerical models. The CPT also provided samples for development of biomarkers.

Batch shake test results were demonstrated to adequately describe sorption and disappearance rate constants in the LAAP soils. Sorption of explosives compounds by the aquifer soils was limited, with all constituents showing K_D values below 1 L kg^{-1} for all soils. These results indicated that a single, average sorption coefficient for each compound in the LAAP soils adequately described sorption for numerical modeling. Disappearance rate constants were low in comparison to those typically observed in surface soils. Use of the disappearance rate coefficients in modeling was complicated by the proximity of the coefficients to zero and the uncertainty that this created about applying results from short-term bench-scale testing to field scale. Use of the disappearance rate coefficients in groundwater models may require adjustment to accurately depict measured groundwater concentrations that reflect field conditions and a longer time frame than is possible with bench-scale batch and column studies. These results suggest that mass-transport limitations rather than site capacity restrict transport at LAAP.

Integration of results from radiorespirometry, nucleic acid, and lipid biomarker techniques was used to evaluate the ability of indigenous microorganisms to degrade explosives. For the two sites, LAAP and JAAP, lipid biomarker technologies provided estimates of viable cell abundance. By identifying the amount and nature of the in situ viable microflora in relation to nitroaromatic contamination, a direct link was established between the rates of contaminant mineralization observed in the radiorespirometry flasks and the indigenous microbial populations. The nucleic acid biomarkers provided the necessary evidence of a genetic capability for natural attenuation at each site. Biomarkers at both sites provided positive evidence that microbial transformation/mineralization processes play a substantial role in explosives attenuation at these sites.

Rates of TNT and RDX mineralization were very low in LAAP soils, and few significant correlations with geochemical parameters and biomarkers were found. At JAAP, mineralization rates were considerably higher. Furthermore, several nucleic acid probes correlated positively with mineralization rates as did the following parameters determined by lipid biomarkers: biomass, abundance of gram-negative, sulfate-reducing and iron-reducing bacteria, and a sulfite reductase. Aerobic degradation of TNT in LAAP soils was indicated by the presence of two catechol oxygenase gene probes. Therefore, the microbial population contained genes for explosives mineralization. At JAAP, presence of a gene for a denitrification enzyme suggested the mechanism for microbial reduction of TNT. Other observed genes supported potential for both anaerobic and aerobic metabolism of the TNT ring.

Biomarker techniques provided an effective tool for demonstrating microbial destruction potential in field samples. The rate and extent of degradation and transformation were also estimated. The effectiveness of microbially mediated natural attenuation depends upon site characteristics, the composition and abundance of the viable biomass, and the genetic capabilities of the site microflora. Biomarkers were effective in measuring each of these at LAAP and JAAP.

The GMS provided efficient numerical tools to integrate and analyze the complex, multidisciplinary field data into simpler graphic forms that were used to illustrate fate and transport of the contaminant plumes. The measured and simulated flow data indicated slow subsurface flow at the LAAP site, because of the low-permeability media and low-hydraulic gradients. The TNT and RDX plumes are virtually static. The simulated flow directions are consistent with the direction of explosives plume propagation. The simulated results indicated that explosives at LAAP may be reduced naturally without posing any threat to offsite receptors. The trigger factors for natural attenuation of explosives at the site including low degree of sorption, low values of the hydraulic conductivity, and low rate of mineralization were evaluated and illustrated. Even though the reduction process is very slow, the plume is confined to a limited area and is not moving significantly. The results of contaminant-mass calculations indicated that the initial mass of TNT and RDX was reduced significantly during 20 years of simulation. The sensitivity analysis suggested that the important model input parameters are the adsorption coefficient rates

and the bio-decay rates. The predicted results should be adjusted and the calibration processes repeated as new data become available.

The three lines of evidence presented by EPA guidance were demonstrated for LAAP as follows: (a) A "clear meaningful trend of declining contaminant mass and/or concentrations at appropriate monitoring or sampling points" was demonstrated by careful monitoring of contamination over a 2-year period. (b) While geochemical data did not "indirectly demonstrate the type(s) of natural attenuation processes active at the site," modeling of site hydrogeology demonstrated that attenuation is expected to continue. Results of biomarker research indirectly demonstrated microbial attenuation processes and the rate at which reduction in contamination may occur. (c) The biomarker results also provided "data from field and microcosm studies (conducted in or with actual contaminated site media) which directly demonstrate the occurrence of a particular natural attenuation process at the site and its ability to degrade the contaminants of concern." Results of this study demonstrate that natural attenuation is a viable option that should be among the options considered for remediation of explosives-contaminated sites.

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13. ABSTRACT (*Maximum 200 words*)

Natural attenuation as a remedial alternative may be appropriate where natural processes are sufficient to protect receptors of concern. The objectives of this project were to demonstrate natural attenuation of explosives at an Army site, optimize groundwater monitoring procedures to generate reliable trends in explosives concentrations over time, evaluate the significance of site capacity on the ultimate fate and transport at the site, apply biomarkers and stable isotopes as monitoring tools, use models for contaminant plume definition and predictions of future contaminant extent, and develop a protocol for selection and implementation of natural attenuation of explosives. The field demonstration was conducted at the Louisiana Army Ammunition Plant and validated at Joliet Army Ammunition Plant. The demonstration included groundwater monitoring and modeling, a cone penetrometry sampling event to characterize site lithology and to obtain sample material for other parts of the study. Results demonstrated declining concentrations of explosives in groundwater over 2 years. The model predicted a shrinking plume over 20 years. Biomarkers demonstrated the microbial degradation potential of RDX and TNT in aquifer soils. Results demonstrated that natural attenuation is a viable option that should be among the options considered for remediation of explosives-contaminated sites.

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Explosives	Intrinsic remediation	Soil partitioning	16. PRICE CODE
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